Tetrahydropyranyl and Tetrahydrofuranyl as New Backbone Protecting Groups For Enhanced Fmoc SPPS

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Introduction

9-Fluorenylmethyloxycarbonyl (Fmoc) solid-phase peptide synthesis (SPPS) is the most reliable method for preparing a wide range of peptide targets. However, synthetic efficiency often declines as chain elongation proceeds, making it difficult to routinely obtain pure peptides with >40 residues. This loss of efficiency is caused by poor chain solubility and aggregation via β-sheet formation (Figure 1a).[1]

Acid-labile amide backbone protecting groups (PGs), such as the 2,4-dimethoxybenzyl (Dmb)[2] group, improve peptide chain solubility and disrupt hydrogen bonding networks (Figure 1b), which can significantly improve crude purity. However, benzyl-based protecting groups are often difficult to remove and the benzyl cations that form during trifluoroacetic acid (TFA) cleavages can alkylate sensitive side chains; this limits their use because multiple protecting groups are needed for the synthesis of longer sequences. Pseudoproline[3] and *N*,*O*-benzylidene[4] dipeptides avoid this deprotection issue, although they are largely limited to serine and threonine containing peptides.

Ideally, backbone protecting groups should be highly acid-labile and easily incorporated into a broad range of amino acid residues. Therefore, we have evaluated the use of tetrahydropyranyl (THP)[5] and tetrahydrofuranyl (THF) groups as backbone protection for Fmoc SPPS, introduced via prepared dipeptide building blocks.

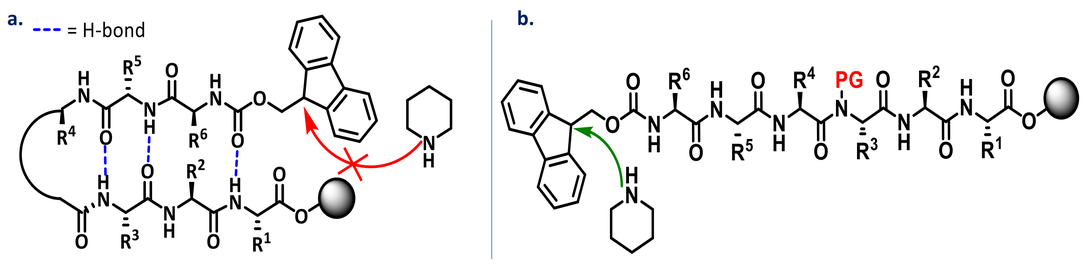


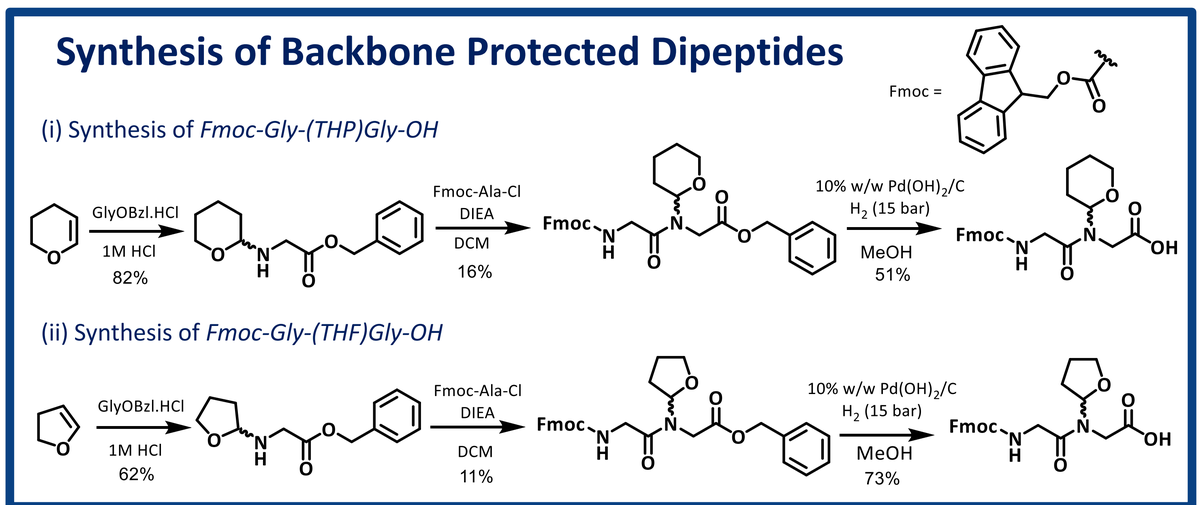
Figure 1. (a.) Backbone hydrogen bonding can cause steric crowding of the N-terminus during Fmoc SPPS. **(b.)** Backbone protection improves solubility and blocks hydrogen bonding, improving synthetic yields.

Objectives

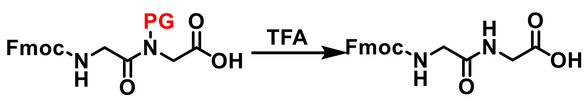
To address these limitations, we are developing new highly acid-labile backbone protecting groups that can be introduced via dipeptide precursors. Our objectives are:

- To explore the synthesis of THP and THF protected dipeptides.
- To investigate the kinetics of these protecting groups in TFA mediated cleavages.
- To evaluate their effectiveness in improving crude peptide purity during SPPS.

Synthesis of Backbone Protected Dipeptides



Acidic Cleavage Deprotection Kinetics



THP and THF protected dipeptides with general structure Fmoc-Gly-(PG)Gly-OH, were reacted in cleavage solutions containing various amounts of TFA, monitored over a 3-hour period. They were compared to the Dmb group and a pseudoproline (Ser($\psi^{Me,Me}$Pro))* motif.

Results:

- Tetrahydrofuran (THF) is hyper acid-labile
- Tetrahydropyran (THP) can be removed in mild cleavage cocktails
- Order of reactivity; THF > $\psi^{Me,Me}$Pro > THP > Dmb

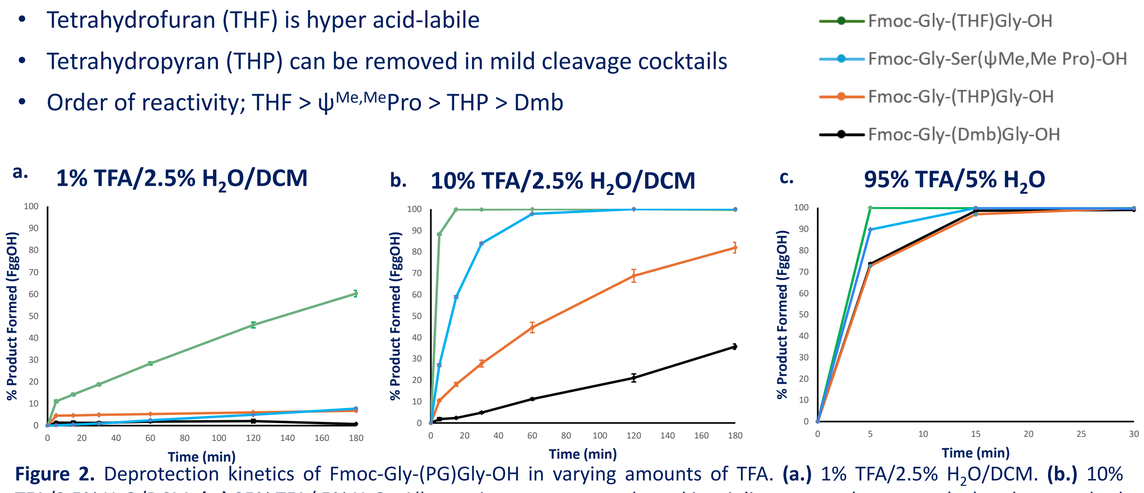


Figure 2. Deprotection kinetics of Fmoc-Gly-(PG)Gly-OH in varying amounts of TFA. **(a.)** 1% TFA/2.5% H_2O/DCM. **(b.)** 10% TFA/2.5% H_2O/DCM. **(c.)** 95% TFA/ 5% H_2O. All experiments were conducted in triplicate; error bars are calculated as standard error. * The pseudoproline was tested in the sequence Fmoc-Gly-(Ser($\psi^{Me,Me}$Pro)-OH.

SPPS of Amyloid Aβ(32-43): Fmoc-IGLMVG(PG)GVVIAT-OH

The dipeptide building blocks (Fmoc-Gly-(PG)Gly-OH) were incorporated in the SPPS of a fragment of amyloid Aβ protein (Figure 3.) and their improvement on the crude product purity was compared to an unprotected dipeptide control (Fmoc-Gly-Gly-OH).

Results:

- THP and Dmb provide equal improvement to crude product purity.
- THF was worse, presenting more deletion sequences closer to the site of protection.
- All the protected dipeptides provided improvement over the unprotected control.

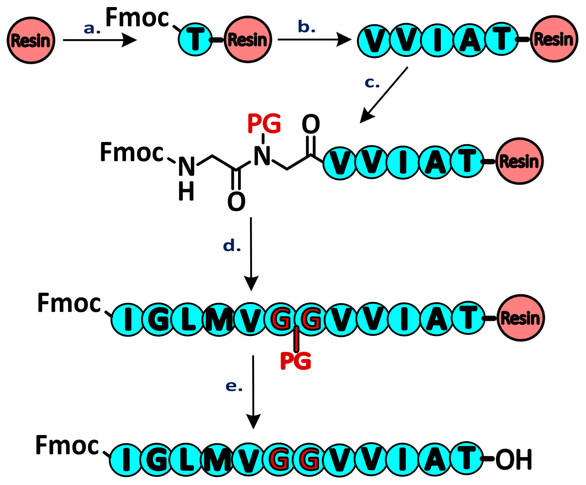


Figure 3. SPPS of amyloid Aβ (32-43). **(a.)** Loading Wang resin; 4 eq. Fmoc-Thr(OtBu)-OH, 6 eq. DIC, 0.1 eq. DMAP, DCM, 4hrs. **(b.)** Standard SPPS conditions; Fmoc deprotection (20% piperidine/DMF, 15 mins), then coupling (4 eq. Fmoc-Xxx-OH, 4eq. HATU, 8eq. DIEA.) **(c.)** Protected dipeptide coupling. (2 eq. Fmoc-Gly-(PG)Gly-OH, 2eq. HATU, 4eq. DIEA.) **(d.)** Standard SPPS conditions. **(e.)** Resin cleabage; 95% TFA / 2.5% H_2O / 2.5% TIPS

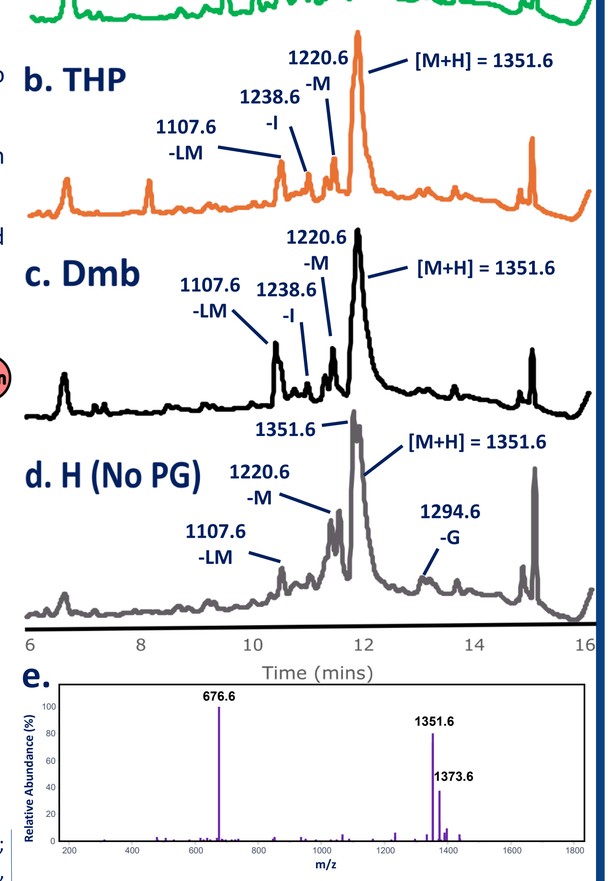


Figure 4. LCMS trace of Fmoc-IGLMVGGVVIAT-OH after SPPS. Dipeptides used are; **(a.)** Fmoc-Gly-(THF)Gly-OH. **(b.)** Fmoc-Gly-(THP)Gly-OH, **(c.)** Fmoc-Gly-(Dmb)Gly-OH, **(d.)** Fmoc-Gly-Gly-OH. **(e.)** Mass spectrum of target peak, [M+H] = 1351.6.

Conclusion

Backbone protected dipeptide building blocks are an effective way to introduce tertiary amide bonds during the SPPS of aggregation-prone sequences and provide an extended solubilising effect that greatly improves crude peptide purity. For the protecting groups investigated it was found that:

- both the THP and THF groups are exceptionally acid-labile.
- they both provide an improvement to the crude product purity during SPPS.
- the THP group can provide the same improvement to crude product purity as Dmb during SPPS.
- the THF performed worse than THP during the SPPS of Aβ(32-43).

Future Work

- Exploring higher yielding synthetic routes of the THP and THF protected dipeptides.
- Incorporating protecting groups into different dipeptide structures.
  (i.e. Fmoc-Xxx-(PG)Gly-OH or Fmoc-Xxx-(PG)Ala-OH)
- Further evaluation of side reactions occurring during SPPS.
  (epimerisation or diketopiperazine formation)

References

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