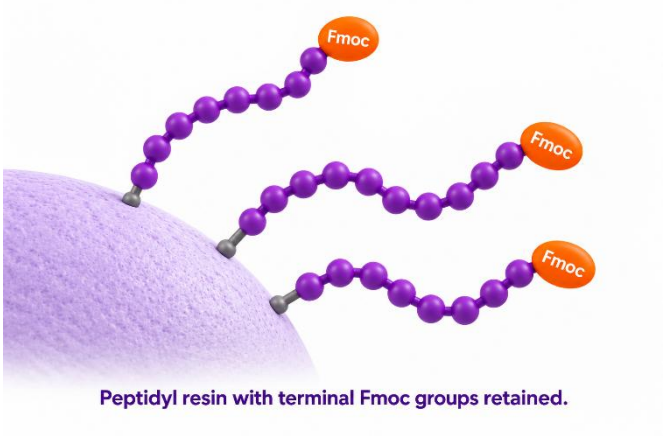
Application Note 85:

Preserving terminal Fmoc groups during storage after Fast-Flow SPPS



Peptidyl resin with terminal Fmoc groups retained.

Abstract

Retention of the terminal Fmoc group is useful for split-and-pool synthesis, analytical workflows, selective side-chain functionalisation and the preparation of protected peptide intermediates for subsequent coupling. Omitting the final deprotection step does not, by itself, ensure that the protecting group remains intact during storage.

Post-synthesis washing was investigated using an NG model dipeptide on the Vapourtec Peptide-Builder™. After 17 hours at 55 °C, a DMF wash gave only 1.7 % Fmoc remaining. A 16 mL wash with DMF containing 0.45 M Oxyma gave 96.6 % Fmoc remaining, while an extended DCM wash gave 95.4 %. These results show that an appropriate final wash can preserve terminal Fmoc protection under the storage conditions tested.

For more details, please contact:
Vapourtec Application Support
application.support@vapourtec.com or call:
+44 (0) 1284 728659

Background

In Fmoc solid-phase peptide synthesis, the terminal protecting group is usually removed at the end of the synthesis. Some applications require it to be retained. In split-and-pool work, protected peptidyl resin may be held between synthesis stages before further chain extension. Retaining Fmoc can also be useful for analytical workflows that require an Fmoc-protected product. With a compatible orthogonal protection strategy, retaining Fmoc protects the N-terminal amine during selective side-chain functionalisation, such as labelling or lipidation at a lysine residue. It also provides protected peptide intermediates for subsequent fragment coupling, allowing the N-terminal amine to be exposed when required.

Unexpected loss of terminal Fmoc during overnight storage therefore changes the material available for subsequent processing. Residual solvent within the resin bed is a potential contributor. The working hypothesis in this study was that trace dimethylamine associated with residual N,N-dimethylformamide (DMF) promotes slow, base-mediated Fmoc cleavage. The wash

strategy was investigated both to displace DMF with dichloromethane (DCM) and to suppress deprotection using Oxyma in DMF.

Experimental approach

A simple NG dipeptide was synthesized on TentaGel resin, with the final Fmoc group retained. The initial Fmoc content was quantified using a controlled glycine coupling followed by controlled deprotection, with the released Fmoc-derived species monitored by UV absorbance at 365 nm. The area under the deprotection curve was calculated to provide a reference value. Post-synthesis wash solvent, volume and flow rate were varied, and following storage of the dried resin in a cartridge, the remaining Fmoc was quantified using the same controlled deprotection procedure. Fmoc retention was expressed as the integrated UV absorbance after storage as a percentage of the initial reference value.

To evaluate Fmoc stability during storage, resin cartridges were held for 17 hours at 55 °C as an accelerated test of post-synthesis storage stability. These conditions provided a consistent basis for comparing the effectiveness of different wash treatments. Additional experiments examined storage at room temperature and under refrigeration.

Results and Discussion

Table 1 summarises the effect of washing and storage conditions on the reported Fmoc content. All stored samples were held for 17 hours; one DMF-washed sample was measured immediately after synthesis.

Wash treatment	Volume (mL)	Flow rate (mL/min)	Storage condition	Fmoc remaining (%)
DMF	Not specified	Not specified	Immediate	89.0
DMF	Not specified	Not specified	Room temperature	60.3
DMF	Not specified	Not specified	55 °C	1.7
DCM	32	4.8	Refrigerated	98.0
DCM	32	4.8	Room temperature	77.6
DCM	16	4.8	55 °C	9.7
DCM	32	4.8	55 °C	50.5
DCM	96	4.8	55 °C	74.5
DCM	96	2.0	55 °C	95.4
DMF with 0.45 M Oxyma	16	4.8	55 °C	96.6
DCM → DMF with 0.45 M Oxyma → DCM	12.9 → 12.7 → 12.9	4.8	55 °C	94.5

Table 1. Fmoc remaining after post-synthesis washing and storage. Sequential wash volumes are listed in delivery order.

DMF-washed resin showed substantial Fmoc loss during storage, with 60.3 % remaining at room temperature and only 1.7 % at 55 °C. Temperature also affected DCM-washed resin: the same 32 mL wash gave 98.0 % Fmoc remaining after refrigeration, compared with 77.6 % at room temperature and 50.5 % at 55 °C.

At 55 °C, increasing the DCM wash volume from 16 to 96 mL at 4.8 mL/min increased Fmoc remaining from 9.7 % to 74.5 %. Reducing the flow rate for the 96 mL wash to 2.0 mL/min increased the result to 95.4 %. The benefit of longer solvent contact is consistent with diffusion-limited solvent exchange within the swollen resin. The high Fmoc retention achieved after the 96 mL DCM wash at 2.0 mL/min also provides indirect evidence that this washing procedure is highly effective at removing residual DMF, although the residual DMF concentration was not measured directly.

Oxyma enables high Fmoc retention with less washing

A 16 mL wash with DMF containing 0.45 M Oxyma gave 96.6 % Fmoc remaining after 17 hours at 55 °C. This was numerically similar to the 95.4 % obtained using 96 mL DCM at 2.0 mL/min, with one-sixth of the wash volume. The calculated solvent delivery time was approximately 3.3 minutes for the DMF/Oxyma wash, compared with 48 minutes for the extended DCM wash. These times exclude other process steps.

A sequence of 12.9 mL DCM, 12.7 mL DMF containing 0.45 M Oxyma, and 12.9 mL DCM gave 94.5 % Fmoc remaining. This treatment used 38.5 mL total solvent and ended with DCM. It therefore offers an alternative when a final DCM wash is preferred, using substantially less solvent than the extended DCM-only treatment.

The Oxyma result indicates that complete DMF removal is not essential for high Fmoc retention under these conditions. Oxyma may reduce the activity of residual dimethylamine through an acid–base interaction. This mechanism remains a hypothesis: the wash comparison demonstrates improved Fmoc preservation, but does not directly establish the source or concentration of the species responsible for deprotection.

Practical guidance for retaining terminal Fmoc

The results support the following approach when preparing Fmoc-protected peptidyl resin for a pause in synthesis or subsequent analysis:

1. Omit the final Fmoc deprotection step and select an appropriate protective wash. The 16 mL DMF wash containing 0.45 M Oxyma used the least solvent among the treatments giving high Fmoc retention after storage at 55 °C.
2. Where a final DCM wash is required, consider the tested DCM–DMF/Oxyma–DCM sequence. Extended DCM washing at a lower flow rate is another effective option, although it requires more solvent and time.
3. Check the entire end-of-synthesis sequence. A cleaning or cooling step that introduces plain DMF after the protective wash can change the final resin environment. In the Peptide-Builder configuration investigated, setting the DCM wash to 58 °C avoided the automatic DMF cooling sequence triggered above 60 °C.
4. Confirm Fmoc retention for the intended peptide, resin and storage conditions before applying the wash to a split-and-pool workflow. The quantitative comparison used an NG model dipeptide and a 17-hour storage period.

To learn more about the Vapourtec system visit www.vapourtec.com

Conclusion

The final wash has a major effect on terminal Fmoc stability during storage after Fast-Flow SPPS. A short wash with DMF containing 0.45 M Oxyma gave 96.6 % Fmoc remaining after 17 hours at 55 °C, compared with 1.7 % following a DMF-only wash. Extended DCM washing at a reduced flow rate and a combined DCM–DMF/Oxyma–DCM sequence also gave high Fmoc retention. These approaches provide practical starting conditions for preserving protected peptidyl resin for split-and-pool work, analytical applications and further synthetic transformations.

To learn more about the Vapourtec system visit www.vapourtec.com