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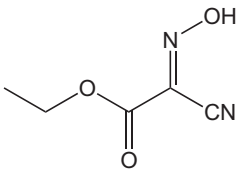
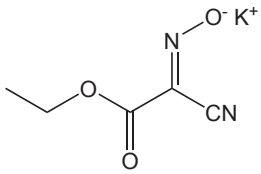
Applications of Oxyma Pure and K-Oxyma Pure in Fmoc SPPS

Oxyma Pure/K-Oxyma Pure





Until recently, 1-hydroxybenzotriazole (HOBt) was the most commonly used auxillary nucleophile for carbodiimide mediated coupling reactions owing to the excellent reactivity and chiral stability of benzotriazolyl (OBt) esters of amino acids and peptides [1]. However, the reclassification of HOBt monohydrate, the standard form of this reagent, as a desensitivized explosive [2] has led to a search for suitable alternatives.

The strongest candidate to emerge is Oxyma Pure, ethyl 2-cyano-2-(hydroxyimino)acetate. It was first identified as a potential coupling additive in the 1970s [3, 4], but a recent re-evaluation by Albericio and coworkers [5] has seen it emerge as one of the most versatile tools for peptide synthesis.

Oxyma Pure has a pKa of 4.60, the same as HOBt, but lacks the potentially explosive triazole structure of HOBt and analogous compounds such as HOAt [6] and HO-6-ClBt [7]. It has been have shown to be more effective and give lower racemization than HOBt in carbodiimide-mediated coupling reactions and help prevent insertion side-reactions at couplings to proline residues. The combination of DIPCDI/Oxyma Pure activation with microwave heating appears to be particularly efficacious [8]. Inclusion of Oxyma Pure in the Fmoc deprotection reagent has been found to significantly reduce aspartimide formation in vulnerable sequences [9]. Addition of Oxyma Pure to acetic anhydride capping reagents leads to marked improvements in capping efficiency, in analogy to HOBt.

Recently, the potassium salt of Oxyma Pure has been introduced (K-Oxyma Pure) [10]. This material has higher solubility than Oxyma Pure in organic and aqueous solvents and does not promote premature cleavage of peptides from highly-acid labile trityl-based resins.

In this Innovation, we review these applications of Oxyma supplemented with work from our laboratories.

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Coupling

Oxyma Pure can be used in an identical manner to HOBt in carbodiimide mediated couplings. Oxyma Pure can be dissolved in DMF and used as a solution on automated synthesizers in place of the standard HOBt/DMF solution. The instrument can be programmed to deliver Oxyma Pure/DMF and DIPCDI in either DMF or DCM to the amino acid derivative, and mixture allowed to preactivate for 2 - 10 minutes before the activated amino acid solution is transferred to the reaction vessel containing the resin.

On synthesizers employing microwave heating, delivery of DIPCDI and Oxyma Pure directly to the reactor, without pre-activation has been found to be effective.

In comparative studies, Subiros-Funosas, *et al*. [5] found Oxyma Pure/DIPCDI activation gave consistently better results than HOBt/DIPCDI and in some cases as good or if not better results than HOAt/DIPCDI. In our hands, almost identical results were obtained between using Oxyma Pure and HOBt as auxiliary nucleophile in the synthesis of the ABRF test peptide.

Application 1: Synthesis of ABRF peptide

H-Lys-His-Asp-Pro-Cys-Gly-Trp-Asn-Gly-Pro-Arg-Pro-Met-Arg-Gly-NH_2 was prepared on a PTI Symphony using 4-fold excesses of Fmoc-amino acids coupled for one hour. Activation was carried out by delivering 0.3 M solutions in DMF of Fmoc-amino acids and Oxyma Pure or HOBt to the peptidyl resin, followed by 0.3 M DIPCDI in DMF. Following cleavage and isolation the crude peptides were analyzed by HPLC. The results obtained using HOBt and Oxyma Pure were essentially identical.

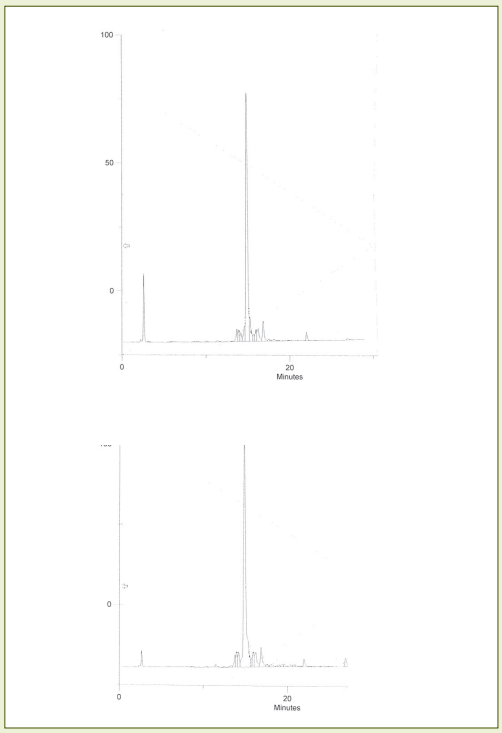

Fig. 1: HPLC profiles of crude ABRF peptide prepared with a) HOBt/DIPCDI and b) Oxyma Pure/DIPCDI.

Aspartimide formation

Despite recent advances in stepwise Fmoc SPPS, base-mediated aspartimide formation remains problematic, particularly for the synthesis of long peptides because of the repeated exposure to piperidine [11]. This side-reaction results in formation of β-aspartyl peptides, together with α- and β-piperidides, and epimerization of the aspartyl residue (Figure 2).

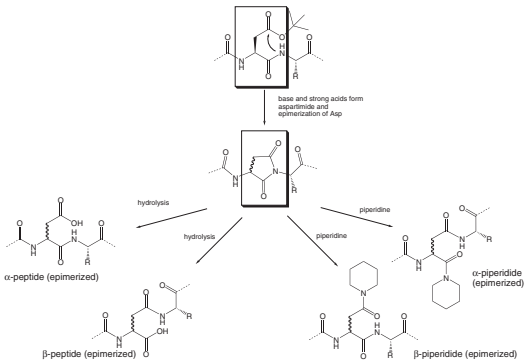

Fig. 2: Aspartimide formation.

Recently, addition of 1M Oxyma Pure to the 20% piperidine in DMF used for Fmoc removal has been shown to be effective in suppressing aspartimide formation [9], presumably by reducing the ionization of the Asp-Xxx amide bond. To demonstrate the effectiveness of this approach, we prepared using the well know VKDGYI and VKDNYI model peptides, containing aspartimide prone Asp-Gly and Asp-Asn sequences. We exposed the peptidyl resins to 20% piperidine or 1M Oxyma Pure in 20% piperidine for 18 h, to simulate the effects of approximately 120 deprotection cycles.

As can be seen from the results in Figures 3 & 4 and Table 1, addition of Oxyma Pure has a marked effect on levels of aspartimide formation. In the case of the peptide containing the particularly sensitive Asp-Gly, substantial aspartimide formation still occurred, indicating this approach is not a practical solution for peptides containing this motif. In such cases, the use of Fmoc-Asp(OtBu)-(Dmb)Gly-OH [12] should be employed which offers total protection.

Table 1: Total aspartimide related by-products formed after 18 h treatment.

Peptide	Piperidine	Piperidine + Oxyma Pure
VKDGYI	92	39
VKDNYI	84	14

For the peptide containing the Asp-Asn sequence (which is the most sensitive case after Asp-Gly), the protection offered appears to be sufficient for short and medium sized peptides (Table 1) However, it is important to consider that for aggregated sequences employing such high concentrations of Oxyma Pure in the piperidine reagent may result incomplete Fmoc removal.

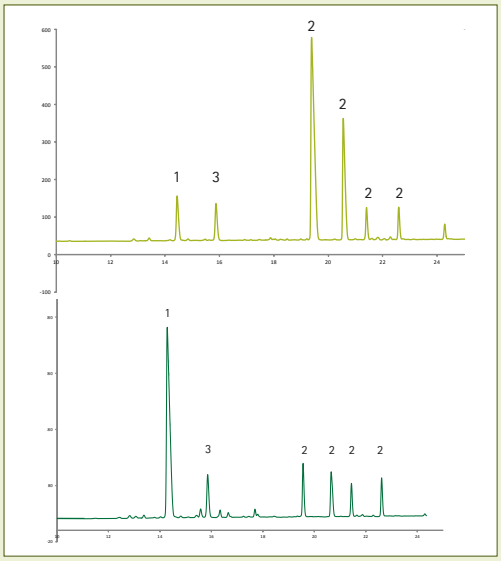

Fig. 3: HPLC profiles of H-Val-Lys(Boc)-Asp(OtBu)-Gly-Tyr(tBu)-Ile-Rink Amide resin treated with a) 20% piperidine in DMF and b) 1M Oxyma Pure in 20% piperidine in DMF for 18 h. 1: Product; 2: piperidides; 3: aspartimide.

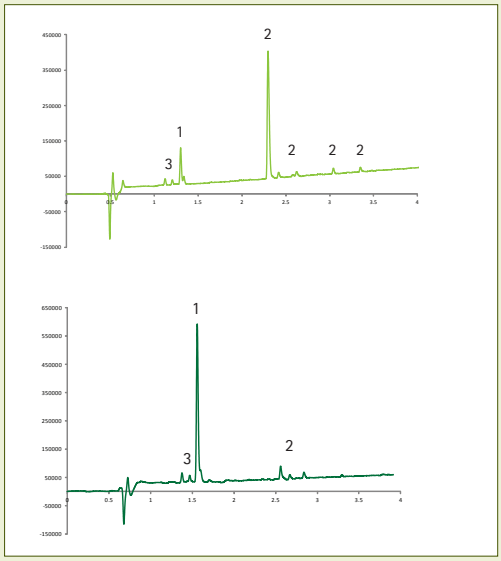

Fig. 4: HPLC profiles of H-Val-Lys(Boc)-Asp(OtBu)-Asn(Trt)-Tyr(tBu)-Ile-Rink Amide resin treated with a) 20% piperidine in DMF and b) 1M Oxyma Pure in 20% piperidine in DMF for 18 h. 1: Product; 2: piperidides; 3: aspartimide.

Capping

Capping of unreacted amino groups following each coupling step during step-wise solid phase synthesis is a very helpful strategy controlling the progress of peptide assembly and for aiding product purification. MALDI-TOF analysis of the crude peptide provides a snapshot of how well the synthesis went. The ladder of very small ions due to capped truncated peptides enables the progress of the assembly to visualized residue by residue in the MALDI-TOF MS spectrum. (ESI-MS is less useful here as the multicharged ions for the product can be often masked by the large number of singularly charged ions of the truncated peptides.) Larger peaks indicate failed couplings and can be used to locate problem regions of the peptides for optimization in subsequent re-synthesis.

Without capping, the by-products arising from incomplete reactions will be very similar to the target peptide and therefore very difficult to remove by RP-HPLC. By employing capping such by-products will be much shorter so it allows the target peptide to be isolated by either gel-fitration or by using chemoselective purification tags, such as the IMAC Tag (see Innovations 03/12).

Different methods for capping have been evaluated using acyl carrier protein (65 - 74) as a model. Treatment for 10 minutes with a 5-fold excess of 0.5 M Ac_2O, 0.125 M DIPEA, 0. 015 M Oxyma Pure in DMF was found to be one of the most effective of the methods tested (Figure 5).

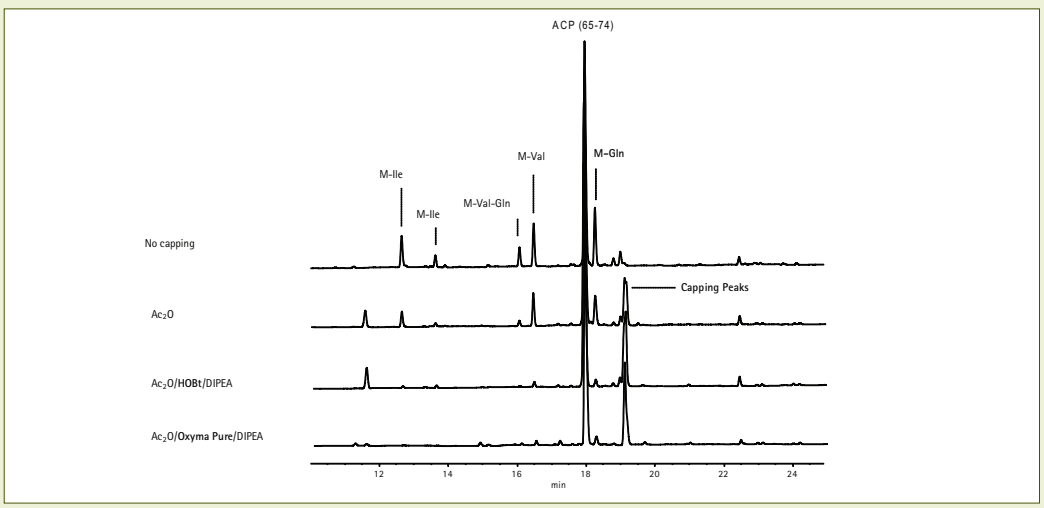

Fig. 5: HPLC profiles of ACP (65-74) prepared without capping and with Ac_2O, Ac_2O/HOBt/DIPEA and Ac_2O/Oxyma Pure/DIPEA capping.

Ordering Information

Cat.No.	Product	Contents	Price EUR
851086	Oxyma Pure	25 g	25.00
		100 g	75.00
851212	K-Oxyma Pure	25 g	25.00
NEW		100 g	75.00
852115	Fmoc-Asp(OtBu)-(Dmb)Gly-OH	1 g	165.00
		5 g	680.00

References

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