

# Novabiochem®

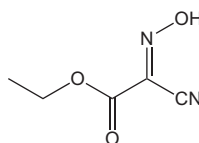
## Letters: 1/09

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### Non-explosive replacement for HOBt

Oxyma Pure



### Features & Benefits

- Non-explosive replacement for HOBt
- Can be used in place of HOBt in carbodiimide-mediated coupling reactions without change of protocol
- Gives results comparable to HOAt in step-wise solid phase synthesis
- Less epimerization than HOBt in fragment condensation reactions

1-Hydroxybenzotriazole (HOBt) [1] has been for decades the most commonly used reagents in peptide synthesis, owing to the excellent reactivity and chiral stability of benzotriazolyl (Bt) esters of amino acids and peptides. Unfortunately, HOBt monohydrate, the standard form of this reagent was recently reclassified by the United Nations as a desensitized explosive [2], with the result that this material can no longer be shipped economically. This loss of ready access to HOBt has caused enormous difficulties to peptide chemists throughout the world and led to a search for a non-explosive alternative to HOBt.

Novabiochem is therefore very pleased to announce that the search is over with the introduction of Oxyma Pure, ethyl 2-cyano-2-(hydroxyimino)acetate, which reacts with protected amino acids in the presence of carbodiimides to form the corresponding active ester (Figure 1).

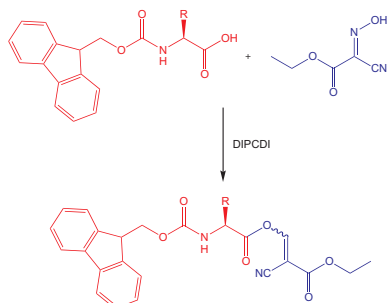


Fig. 1: Coupling with Oxyma Pure.

Oxyma Pure was first identified as a potential coupling additive in the 1970s [3, 4]. It 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 [5] and Cl-HOBt [6]. These properties led Albericio and co-workers [7, 8] to undertake an exhaustive evaluation of Oxyma Pure as the replacement for HOBt. In the coupling of the notoriously racemisation prone Z-Phg-OH to H-Pro-NH<sub>2</sub> by DIPCPI, they found that the use of Oxyma Pure led to formation of only 1.1% DL Z-Phg-Pro-NH<sub>2</sub>, compared with 3.3 and 9.3% for HOAt and HOBt respectively. Similarly, in the fragment coupling of Z-Phe-Val-OH to H-Pro-NH<sub>2</sub>, the use of Oxyma Pure/DIPCPI led to only 3.8% epimerization compared to 8.9% when HOBt/DIPCPI was used. In solid phase synthesis of ACP and enkephalin analogs containing MeAla and Aib residues in place of Gly residues, Oxyma Pure/DIPCPI activation gave consistently better results than HOBt/DIPCPI and in some cases as good or if not better results than HOAt/DIPCPI.

In practice, 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 DIPCPI 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. A manual protocol for using Oxyma Pure is given in Method 1.

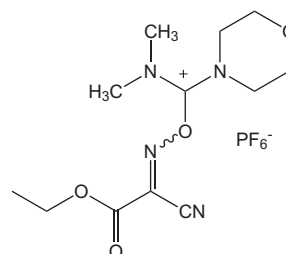
#### Method 1: Coupling using Oxyma Pure

1. Dissolve protected amino acid (4 eq.<sup>a</sup>) and Oxyma Pure (4 eq.<sup>a</sup>) in DMF.
2. Add DIPCPI (4.4 eq.<sup>a</sup>) and agitate for 10 min.
3. Add solution to peptidyl resin.

<sup>a</sup>relative to resin loading

## NEW Coupling reagents

### COMU



#### Features & Benefits

- Comparable coupling efficiency to HATU
- Mediates coupling with low racemization or epimerization
- Ideal for solution phase synthesis as by-products are water soluble
- Excellent solubility and stability in DMF and NMP
- Likely to have low potential for causing allergic reactions
- Low or non-existent explosivity

COMU [7, 9], a third generation coupling reagent based on the Oxyma Pure leaving group, offers a number of significant advantages over conventional HOBt-based coupling reagents such as HBTU, HCTU and HATU. COMU is significantly less likely to cause allergic reactions such as contact dermatitis or asthma. Differential thermal analysis and ARC studies have shown that COMU is not explosive under normal operating conditions. The by-products of using COMU are water soluble and easily removed, making it an excellent choice of coupling reagent for solution phase peptide synthesis. COMU is more stable and soluble than tetramethylurea-based reagents such as HATU and HBTU. For example, COMU gives a 1.5 M solution in DMF, compared to 0.5 M for HATU and HBTU. Moreover, in open vials, solutions of COMU in DMF were found to still possess 93% activity after 48 hours, whereas those of HBTU and HATU dropped to 76 and 86% respectively. These properties have important practical implications: solutions of COMU can be maintained on automated synthesizers for longer without loss of reactivity; reactions can be performed at higher concentrations with concomitant improvements in efficiency. Finally, and most importantly, the coupling efficiency observed when using COMU is superior to that of HBTU and is comparable, and in many cases superior, to that of HATU. Epimerization during coupling of fragments also appears to be less when using COMU than with HBTU or HATU.

COMU is used in exactly the same way as PyBOP®, HBTU or HATU (Method 2), but instead of generating a benzotriazolyl ester it forms the corresponding ester of Oxyma Pure (Figure 2). In situations where racemization is an issue, COMU can be used with as little as one equivalent of base since the morpholino oxygen acts as an integral base.

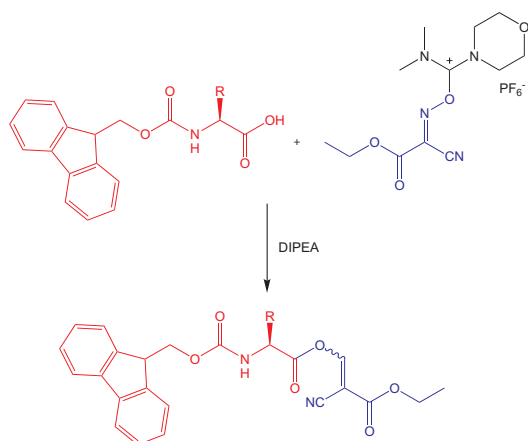


Fig. 2: Coupling with COMU.

### Method 2: Coupling using COMU

1. Dissolve protected amino acid (4 eq.<sup>a</sup>) and COMU (4 eq.<sup>a</sup>) in DMF.
2. Add DIPEA (8 eq.<sup>a</sup>) and add solution to peptidyl resin.

<sup>a</sup>relative to resin loading

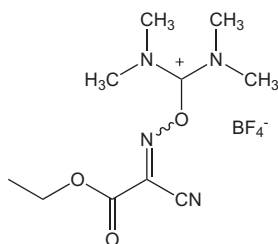
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**NEW**

COMU

5 g  
25 g  
100 g

### TOTU



### Features & Benefits

- Ideal for solution phase synthesis as by-products are water soluble
- Likely to have low potential for causing allergic reactions
- Excellent solubility and stability in DMF and NMP
- Low or non-existent explosivity

TOTU is the original coupling reagent based on ethyl 2-cyano-2-(hydroxyimino)acetate (Oxyma Pure) developed by Breipohl and König [10]. It exhibits many of the excellent properties of COMU: high reactivity and low explosivity, and is likely to have low potential to cause allergic reactions. Like COMU, the by-products are water soluble making it an ideal reagent for solution phase peptide synthesis.

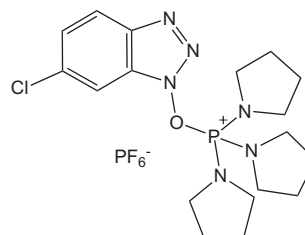
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**NEW**

TOTU

5 g  
25 g  
100 g

### PyCloCk



### Features & Benefits

- Generates Cl-Bt esters that are more reactive than Bt esters
- Unlike HCTU, does not cause guanidinylation
- Ideal for difficult and hindered coupling reactions

PyCloCk is the 6-chloro analog of PyBOP. Reaction of protected amino acids with PyCloCk in the presence of base generates the corresponding 6-chloro-1-benzotriazolyl (Cl-OBt) ester [6]. These active esters are considerably more reactive than those produced when using PyBOP or HBTU, owing to 6-chloro-1-hydroxybenzotriazole (Cl-HOBt) being more acidic than HOBt. PyCloCk is an excellent coupling reagent for situations where carboxyl activation may be sluggish, such as cyclizations and fragment condensations, as unlike imminium-based reagents such as HBTU and HATU excess PyCloCk cannot cause end-capping of peptide chains.

851087

**NEW**

PyCloCk

5 g  
25 g  
100 g

### Novabiochem®'s other coupling reagents

01-62-0001	BOP	5 g 25 g 100 g
01-62-0041	HATU	5 g 25 g
01-62-0010	HBTU	5 g 25 g 100 g
01-62-0038	HCTU	5 g 25 g 100 g
01-62-0021	MSNT	1 g 5 g 25 g
01-62-0016	PyBOP®	5 g 25 g 100 g
01-62-0017	PyBrOP®	5 g 25 g 100 g
01-62-0015	TBTU	5 g 25 g 100 g
01-62-0011	WSC	5 g 25 g

# Enhanced specifications for Fmoc-amino acids

All peptide chemists are aware of the need to achieve near quantitative yields during coupling and deprotection reactions in step-wise solid phase synthesis. Equally important, however, is the need to use the highest purity protected amino acids, as truncated and modified peptides arising from small impurities in these reagents can quickly accumulate, dramatically reducing product yield and purity. Novabiochem, therefore, constantly strives to improve the quality of our products, and it is for this reason that we have implemented two additional analytical procedures for controlling the quality of our Fmoc-amino acids.

Firstly, we have now introduced extremely stringent limits on the levels of residual ethyl acetate and acetate, as traces of these materials can give rise to chain terminated by-products during peptide assembly. Secondly, we now analyse for  $\beta$ -alanine contamination, as trace amounts of  $\beta$ -alanine can arise during manufacture of Fmoc-amino acids from the use of Fmoc-OSu *via* the Lössen rearrangement [11, 12]. These additional measures should ensure our customers of the highest quality products for their research and GMP peptide applications.

## References

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