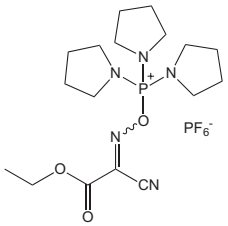
Novabiochem®
Letters: 3/10

Contents

NEW Coupling reagent

PyOxim



Features & Benefits

- Cost effective alternative to COMU
- Mediates coupling with low racemization or epimerization
- Ideal for fragment condensation and synthesis of cyclic peptides as the reagent cannot cause guanidinylation.
- Excellent solubility in DMF and NMP
- Likely to have low potential for causing allergic reactions
- Low or non-existent explosivity

PyOxim [1] is a new coupling reagent that combines the advantages of Oxyma Pure-based reagents with those of phosphonium coupling reagents, making it the ideal choice for both solution and solid phase synthesis.

MERCK

In tests, PyOxim has been shown to be one of the most efficient coupling reagents described to date. During the synthesis of the model peptide H-Tyr-MeLeu-MeLeu-Phe-Leu-NH_2, PyOxim was observed to be superior to PyBOP, HBTU and HCTU, and gave near identical results to COMU (Table 1) [2]. Similarly, Subirós-Funosas, *et al.* [1] found PyOxim to be more effective than PyBOP, PyClocK and PyAOP in the synthesis of a range of Aib-containing test sequences.

Epimerization during coupling of fragments also appears to be less when using PyOxim than with PyBOP, PyClocK or PyAOP. In the coupling of the notoriously racemization prone Z-Phg-OH to H-Pro-NH_2, Subirós-Funosas, *et al.* [1] found that the use of PyOxim led to formation of only 0.3% DL Z-Phg-Pro-NH_2, compared with 5.8%, 2.2%, and 1.6% for PyBOP, PyClocK, and PyAOP, respectively. Similarly, in the solid phase fragment coupling of Z-Gly-Gly-Val-OH to H-Pro-Gly-Gly-NH_2, the use of PyOxim/TMP led to 4.1% epimerization compared to 4.4 % when PyAOP/TMP was used.

Table 1: Synthesis of H-Tyr-MeLeu-MeLeu-Phe-NH_2 using various coupling reagents. Coupling of N-terminal Tyr and MeLeu residues was performed for just 5 mins to emphasize differences in efficiencies between reagents.

% Compostion of products by HPLC					
Sequence	PyBOP	PyOxim	HBTU	HCTU	COMU
MeLFL	60	10	57	31	9
YMeLFL	31	45	31	35	47
MeLMeLFL	5	5	8	9	4
YMeLMeLFL	4	40	4	25	40

In addition to high reactivity, PyOxim offers a number of other significant benefits over conventional uronium-based coupling reagents such as HBTU and HCTU. PyOxim is less likely to cause allergic reactions such as contact dermatitis or asthma and is not explosive under normal operating conditions. It has excellent solubility in DMF, giving a 2.5 M solution compared to 0.5 M for HATU and HBTU [1]. This property has an important practical implication as it facilitates coupling reactions at higher concentrations with concomitant improvements in efficiency. Solutions of PyOxim have good stability in DMF. In a closed vial, a 0.25 M solution of PyOxim were found to still possess 90% activity after 48 hours, whereas those of COMU and PyBOP dropped to 67% and 88% respectively [2].

Finally, being a phosphonium salt, PyOxim cannot cause guandinylation of amino groups like uronium-based reagents [3]. This side reaction is particularly problematic when carboxyl activation is slow, for example in the case of fragment and cyclization reactions, since it leads to formation of truncated peptides bearing a N-terminal guanidino group (Figure 1). Formation of such by-products also causes difficulties in the assembly of long peptides as these short positively charged peptides can mask the presence of the target ion in the ESI mass spectrum.

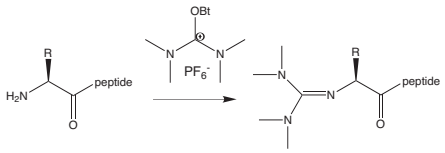


Fig. 1: Guanidinylation by uronium-based coupling reagents.

PyOxim is used in exactly the same way as PyBOP, HBTU or HATU (Method 1), but instead of generating a benzotriazolyl ester it forms the corresponding ester of Oxyma Pure (Figure 2).

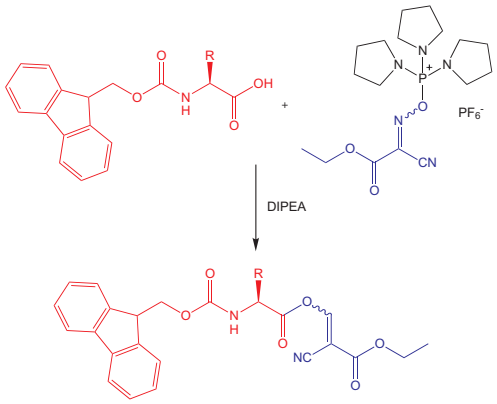


Fig. 2: Coupling with PyOxim.

Method 1: Coupling using PyOxim

1. Dissolve protected amino acid (4 eq.[a]) and PyOxim (4 eq.[a]) in DMF.
2. Add DIPEA (8 eq.[a]) and add solution to peptidyl resin.

[a]relative to resin loading

851095	PyOxim	5 g
NEW		25 g
		100 g

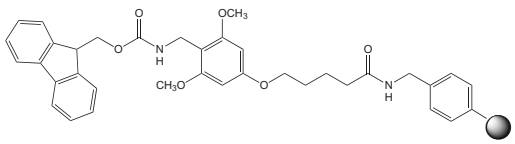
Novabiochem®'s other coupling reagents

851004	BOP	5 g
		25 g
		100 g
851085	COMU	5 g
		25 g
		100 g
851091	DEPBT	5 g
		25 g
		100 g
851013	HATU	5 g
		25 g
851006	HBTU	5 g
		25 g
		100 g
851012	HCTU	5 g
		25 g
		100 g
851011	MSNT	1 g
		5 g
		25 g

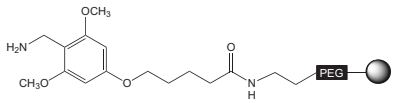
851009	PyBOP®	5 g 25 g 100 g
851010	PyBrOP®	5 g 25 g 100 g
851087	PyClocK	5 g 25 g 100 g
851008	TBTU	5 g 25 g 100 g
851090	TFFH	1 g 5 g 25 g
851088	TOTU	5 g 25 g 100 g
851007	WSC·HCl	5 g 25 g

Resins for Fmoc SPPS of peptide amides

Fmoc-PAL AM resin



PAL NovaSyn TG resin



Features & Benefits

- Resin is loaded with C-terminal residue using standard coupling methods
- Treatment with 95% TFA releases peptide amides
- Gives higher yields in microwave-assisted SPPS than Rink amide-based resins

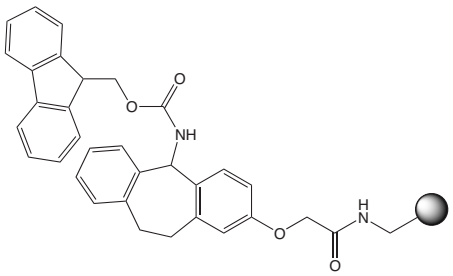
PAL resins are excellent supports for the synthesis of peptide amides by Fmoc SPPS. They consist of Barany's aminomethyl-dimethoxyphenoxyvaleric acid linker [4] attached to aminomethylated polystyrene or NovaSyn® TG resin. The amino group of this linker can be easily acylated under standard coupling conditions. Following peptide assembly, treatment with 95% TFA containing scavengers releases the desired peptide amide. Studies have shown the acid sensitivity of this linker to be around twice that of the Rink amide linker [5]. There is some evidence to suggest that PAL resins give greater yields in microwave assisted synthesis.

855133	Fmoc-PAL AM resin	1 g
NEW		5 g
855137	PAL NovaSyn® TG resin	1 g
NEW		5 g

Novabiochem®'s resins for Fmoc SPPS of peptide amides

855047	NovaPEG Rink Amide resin	1 g 5 g 25 g
855009	NovaSyn® TGR resin	1 g 5 g 25 g
855001	Rink Amide resin (100-200 mesh)	1 g 5 g 25 g
855130	Rink Amide AM resin (100-200 mesh)	1 g 5 g 25 g
855003	Rink Amide MBHA resin (100-200 mesh)	1 g 5 g 25 g
855031	Rink Amide NovaGel™	1 g 5 g 25 g
855016	Rink Amide PEGA resin	1 g 5 g

Ramage Amide AM resin



Features & Benefits

- Resin is loaded with C-terminal residue using standard coupling methods
- Treatment with 3% TFA releases peptide amides
- Useful support for the synthesis of acid-sensitive peptides

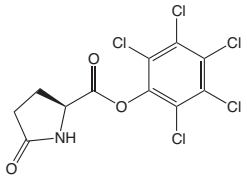
The Novabiochem® brand is pleased to offer Ramage's dibenzocycloheptadiene linker [6] attached to aminomethylated polystyrene. Following Fmoc removal, the resin can be acylated under standard conditions and used in Fmoc SPPS. The linker is considerably more acid sensitive than the Rink amide or PAL linkers. This enables peptide amides to be released from the resin with 3% TFA in DCM and thus makes it a useful tool for the synthesis of acid sensitive peptides or protected peptide fragments.

855134	Ramage Amide AM resin	1 g
NEW		5 g

Novabiochem®'s resins for Fmoc SPPS of protected peptide amides

855008	Sieber Amide resin	1 g 5 g 25 g
855013	NovaSyn® TG Sieber resin	1 g 5 g 25 g

Derivative for synthesis of pyroglutamyl peptides



Features & Benefits

- Useful tool for the introduction of N-terminal pyroglutamyl residues

The use of unprotected pyroglutamic acid in conjunction with modern coupling reagents like HCTU can lead to multiple additions, as the lactam nitrogen can be acylated under forcing conditions. The simplest solution, which avoids the need for deployment of an additional protecting group, is the use of a mildly activated ester such Pyr-OPcp. To use, excess Pyr-OPcp is simply dissolved in DMF with 1 eq. of Oxyma Pure as a catalyst and the solution added to the peptidyl resin. The coupling is usually complete in 1 h.

854193	Pyr-OPcp	1 g
NEW		5 g

New Wall Poster

Guide to the selection of resins for solid phase peptide synthesis

Please order your free copy online at www.merck4biosciences.com

References

1. R. Subirós-Funosas, et al. (2010) *Org. Biomol.Chem*., **8**, 3665.
2. R. Behrendt, et al., Poster 83, Presented at the 31st European peptide Symposium, Copenhagen, Sept 2010.
3. H. Gausepohl, et al. in "Peptides, Chemistry & Biology, Proc. 12th American Peptide Symposium", J. A. Smith & J. E. Rivier (Eds.), ESCOM, Leiden, 1992, pp. 523.
4.. F. Albericio & G. Barany (1987) *Int. J. Peptide Protein Res.*, **30**, *206*
5. M. S. Bernatowicz, et al. (1989) *Tetrahedron Lett.*, **30**, 4645.
6. R. Ramage, et al. (1993) *Tetrahedron Lett.*, **34**, 6599.