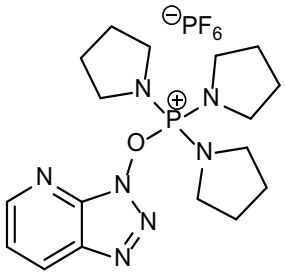
Novabiochem®
Letters 1 Vol. 14

Novabiochem®
30 Years of Innovation

Product focus: Coupling reagents

NEW • PyAOP

Phosphonium-based coupling reagent



Features & Benefits

- Combines the high reactivity of HATU with the lack of side reactions of PyBOP
- Does not cause guanidinylation of free amino groups
- Perfect for fragment condensation and cyclization reactions

Introduction of PyAOP completes our range of phosphonium-based coupling reagents. PyAOP [1] is the phosphonium analog of the uronium-based coupling reagent HATU [2] which is regarded by many as the gold standard in situ coupling reagent for solid phase synthesis. Like HATU, PyAOP mediates coupling reactions, particularly onto secondary and α,α-disubstituted amino acids, with high efficiency and low racemization [3]. However, unlike HATU, PyAOP does not cause side-termination side-reactions [1]. It can therefore be used in excess, and is ideal for mediating cyclization and fragment condensations reaction and for coupling when conversion to the active ester is slow.

NEW • Larger packs sizes for HATU

Gold standard for hindered couplings

HATU combines excellent solution stability and high coupling efficiency, making it is the most popular coupling reagent for hindered and problematic couplings . HATU is now available in 100 g catalog quantities. Bulk quantities are also available on request.

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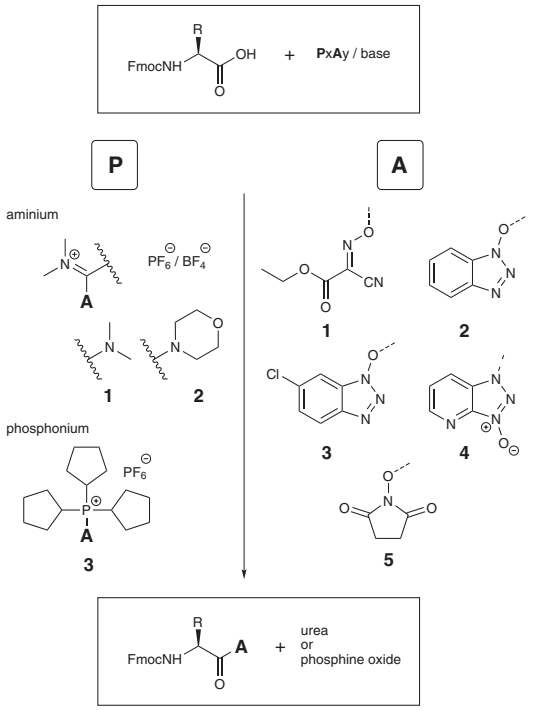
Guide to selection of coupling reagents

The Novabiochem® brand offers one of the most extensive ranges of high-quality coupling reagents for *in situ* activation on the market. However, with such a plethora of reagents available, the choice of the optimum coupling reagent for a particular application is not always straightforward. Condensation reagents vary in terms of coupling efficiency, stability, solubility, or reactivity of active species. To mark the introduction of PyAOP and larger pack sizes for HATU to our portfolio, this Novabiochem® Letters offers an overview of our new and existing coupling reagents, and information to assist your reagent selection.

In-situ activating reagents

In situ activating reagents are widely accepted because they are easy to use, they give fast reactions, even between sterically hindered amino acids, and their use is generally free of side reactions. Most are based on phosphonium or aminium (formerly known as uronium) salts which, in the presence of a tertiary base, can smoothly convert protected amino acids to a variety of activated species (Figure 1). The properties of Novabiochem's coupling reagents are summarized in Table 1.



P1A1 = TOTU , **P1A2** = HBTU/TBTU , **P1A3** = HCTU , **P1A4** = HATU , **P1A5** = TSTU
P2A1 = COMU
P3A1 = PyOxim , **P3A2** = PyBOP , **P3A3** = PyClock , **P3A4** = PyAOP

Fig. 1: Active esters generated with most commonly used coupling reagents.

The most commonly employed reagents, BOP, PyBOP, and HBTU generate OBt esters, and these have found wide application in routine SPPS and solution synthesis for difficult couplings. Coupling reagents are also available which generate esters that are more reactive than OBt. Three such reagents are HATU [2], PyAOP [1, 3], and HCTU [4], PyClocK, which in the presence of base convert carboxylic acids to the corresponding OAt and O-6-ClBt esters respectively. Such esters are more reactive than their OBt counterparts owing to the lower pKa of HOAt and HO-6-ClBt compared to HOBt. Furthermore, HOAt has the added benefit of the pyridine nitrogen which provides anchiomeric assistance to the coupling reaction, making HATU and PyAOP the most efficient coupling reagent of the OBt series.

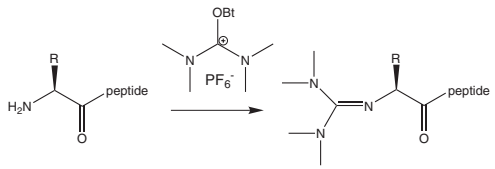
Recently, coupling reagents based on the Oxyma Pure leaving group have been introduced, the most useful of which are COMU [5, 6] and PyOxim [7]. The evidence on the relative reactivity of the Oxyma esters generated by these reagents is unequivocal. The original reports of these Oxyma-based reagents reagents appear more efficient than those based on HOAt, whereas our in-house tests [8] indicate the HOAt reagents to be superior. Nevertheless, Oxyma-based reagents always perform better than those based on HOBt (PyBOP, HBTU) and O-6-ClBt (PyClocK and HCTU). One particular advantage of oxyma-based coupling reagents is that they are not-based on potentially explosive triazole reagents.

In our experiments, the efficency of a coupling reagent seems to be almost entirely related to the nature of the active ester it generates, with an order of reactivity OAt> Oxyma Pure > 2-ClOBt > OBt. The structure of the uronium or phosphonium componet appeared to have little influence.

Phosphonium versus Uronium reagents

With the exception of COMU, solutions of uronium-based reagents in DMF are exceptionally stable, making them ideal for use on synthesizers which utilize pre-made solutions of coupling reagents. In contrast, solutions of phosphonium reagents in DMF have moderate stabilty, and should be kept in closed vials and only used for a maximum of 2 days. However, phosphonium reagents are significantly more soluble in DMF than uronium reagents. This has important practical implications, as it enables reactions to be performed at higher concentrations with concomitant improvements in efficiency.

Phosphonium coupling reagents generally cleaner reactions than uronium reagents. The latter can cause chain termination by guadinylating the *N*-terminal amino group [9]. This side reaction is particularly problematic when carboxyl activation is slow, for example in the case of fragment and cyclization reactions, or if the excess uronium reagent is used. 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. In contrast to uronium reagents, phosphonium reagents can be used in excess and can even be used to "feed" a slow cyclization or fragment coupling



reaction, to help drive them to completion.

Fig. 2: Guanidinylation caused by uronium coupling reagents.

Finally, there are a number of reports describing allergic reactions to uronium-base coupling reactions such as HBTU or HCTU. But there are only few, if any, reports of similar issues with phosphonium-based coupling reagents.

Applications

Uronium	Phosphonium
Synthesizers using stock solutions, particularly if solutions are open to atmosphere	Synthesizers where solutions held in closed containers and reagents consumed rapidly
Scale-up if cost of reagent is a consideration	Scale-up, if reagent concentration is a consideration
Can not be used in excess during fragment condensation and cyclizations	Can be used in excess in fragment condensations and cyclizations

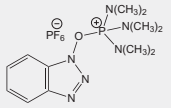
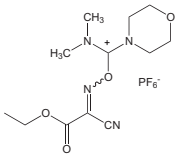
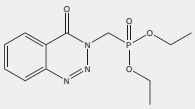
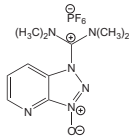
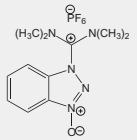
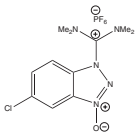
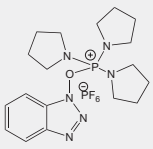
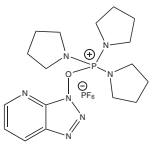
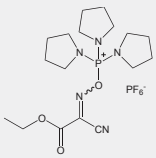
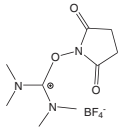
Coupling reagent	Prod. No.	Structure	Solubility (M)	Stability in DMF closed vial	Reactivity of active species	Comments
BOP	851004		>1.5	n/a	4	Water soluble by-products make it useful for solution phase synthesis
COMU	851085		1.5	Low	1/2	High reactivity, possibly superior to HATU for hindered couplings. Non-explosive. Limited solution stability. Causes guanidylation.
DEPBT	851091		n/a	n/a	4	Excellent reagent for coupling of protected fragments with low racemization
HATU	851013		0.45	Excellent	1	Gold standard for hindered couplings, but expensive compared to other reagents
HBTU	851006		0.5	Excellent	4	Excellent reagent for routine synthesis. May cause guanidinylation.
HCTU	851012		0.75	Excellent	3	More reactive and expensive than HBTU. May cause guanidinylation.
PyBOP	851009		>1.5	Moderate. Solutions need making fresh daily	4	Excellent reagent for routine synthesis. Ideal of in situ activation as it does not cause guanidinylation. Clean.
PyAOP	851221		>1.5	Low - Moderate. Solutions need making fresh daily. Best kept under N_2	1	Excellent reagent for hindered couplings, fragment condensation and peptide cyclization. Ideal of in situ activation as it does not cause guanidinylation. Clean.
PyOxim	851095		>1.5	Moderate. Solutions need making fresh daily	1/2	Similar advantages to PyBOP but generates a more reactive active species. Clean.
TSTU	851206		n/a	Excellent	4	Useful for the conversion of dyes/PEG reagents to water compatible OSu esters

Table 1: Properties of commonly used coupling reagents.

Ordering information

Cat.No.	Product	Contents	Price EUR
OBt ester			
851004	BOP	25 g	66.00
		100 g	220.00
851006	HBTU	25 g	40.00
		100 g	120.00
85109	PyBOP	25 g	82.00
		100 g	245.00
851008	TBTU	25 g	40.00
		100 g	120.00
O-6-ClBt			
851012	HCTU	25 g	82.00
		25 g	245.00
851087	PyClocK	25 g	102.00
		100 g	300.00
OAt			
851013	HATU	25 g	300.00
		100 g	600.00
851221	PyAOP	25 g	125.00
NEW		100 g	375.00
Oxyma Pure			
851085	COMU	25 g	125.00
		100 g	375.00
851095	PyOxim	25 g	100.00
		100 g	360.00
851088	TOTU	25 g	102.00
		100 g	300.00

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For more information please contact our local offices:

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For Technical Service contact:
technical@novabiochem.com

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