Green Chemistry: healthier chemists with every peptide synthesized

As solid phase peptide synthesis (SPPS) typically uses an excess of reagents to drive the completion of these reactions, there is a need for the use of a large amount of solvents, most of which are hazardous. In fact, solvents make up the major component of the reaction mixture representing 80−90% of the nonaqueous mass, as concluded in a survey by GSK in 2007 about the materials used for the manufacturing of active pharmaceutical ingredients (APIs).[1]

Benzotriazols (and variants) are frequently used as coupling agents for peptide syntheses in the pharmaceutical industry. However, their safety profile must be carefully considered as these compounds display explosive properties, when heated under defined confinement or when subjected to mechanical stimulus that leads to restrictions for their shipping and handling. In addition, a variety of allergic responses have been reported from exposure to some coupling agents. Moreover, residues of the starting material 1-Chloro-2-nitrobenzene and hydrazine, used for the synthesis of bezotriazol moieties are known to have toxic effects, even at parts per billion (ppb) levels. For example, irritating effects on the mucous membrane, as well as skin irritation, have been reported when exposed to the dust of HOBt.

Taking into consideration the hazardous potential of the essential benzotriazole-based coupling reagents, Luxembourg Biotechnologies decided to reduce the environmental and health impact of these chemicals by developing compounds that are safer yet achieve similar results as HOBt. With these compounds we move towards our goals to create a healthy and safe working environment and achieve a zero disaster risk that is implemented in local policies, training, lab goal setting, and performance evaluation.

Our cutting-edge research, together with Prof. F. Albericio, led to the discovery of various Oxyma-derived coupling reagents that are applied worldwide in peptide synthesis projects. These novel coupling reagents (Table 1), are environmentally friendly and non-allergenic while also driving efficient peptide synthesis reactions.

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Since the discovery of these Oxyma-based reagents, they have been applied towards enhanced coupling efficiency of amino acids for peptide synthesis; Weinreb amides synthesis; Oligobenzamide formation; conjugation of amino groups and more. This class of reagents has the additional benefit of being eco-friendly and as such has been widely applied in the pharmaceutical industry – with significant demand due to the REACH registration in Europe.

Over the last few years, the global market size for peptide therapeutics (for a variety of medical indications e.g. metabolic disorders, infectious diseases and cancers) has been witnessing a substantial rise. Two key factors have led to the significant reduction in the production and purification costs of peptide drugs:

- Advancements in the robustness, reproducibility and purity of automated SPPS
- Development of more efficient, highly potential and cost effective products for peptide coupling, such as OxymaPure®; COMU®; PyOxim® (Table 1)

Cost reduction in peptide synthesis labs has also been realized by reducing the total waste generated (by choosing the appropriate reagents).

In the 1970s, the average frequency of peptides entering clinical trials was around one a year. By 2020, that number will be close to 20, and six peptides received approval from FDA as new molecular entities in 2012. Around 80 approved peptide drugs are on the market and over 500 pre-launch peptide therapeutics are in development across 325 companies, with a continuous annual growth rate of 10%. This explosion in growth continues to put the spotlight on the research and development of green chemistries that increase the efficiency of peptide synthesis, such as the green Oxyma-based reagents.

These new coupling reagents, might be your solution to green production of your new pharmaceutical entity. Inquire about a free sample today (supplies limited) by sending an email to peptides@gyrosproteintech.com.

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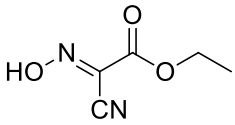
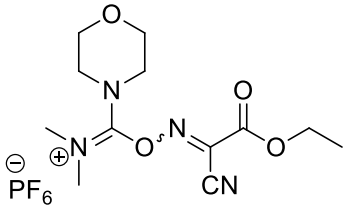
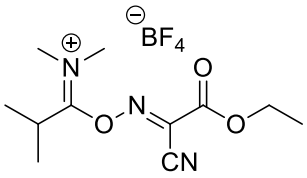
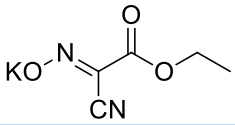
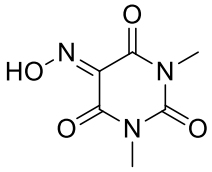
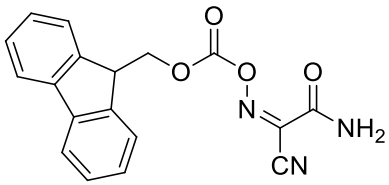
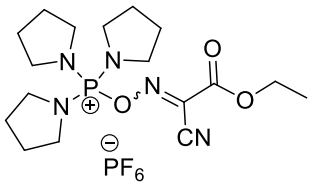
Table 1: Eco-friendly Oxyma-Derived Coupling Reagents

Product identification		Description
Trade Name:	OxymaPure	The acidic nature of cyanooximes has led to the development of activated derivatives for acylation reactions on the basis of their potential as leaving groups. **OxymaPure** offers the best balance between reactivity and stability, in addition to its high solubility in a broad spectrum of solvents. In spite of its early use as an epimerization-suppressing additive, OxymaPure remained unnoticed as a coupling reagent in the ensuing decades. Following the reevaluation of OxymaPure and other acidic oximes by DeGrado's and Albericio's groups, OxymaPure and its derived coupling reagents have emerged as worthy alternatives to benzotriazoles. In a short period of time, this oxime scaffold has rapidly been adopted in research laboratories to effect a broad range of acylations (synthesis of Peptide Bonds; Nucleosides; Weinreb Amide; Oligobenzamide; ester bond and more).[2]
Chemical Name:	Ethyl (hydroxyimino)cyanoacetate	
CAS:	3849-21-6	
Molecular structure:		
Trade Name:	COMU	**COMU** was constructed as a third generation uronium salt. COMU combines a morpholonium-based iminium moiety as proton acceptor, and OxymaPure® as leaving group to provide a superior and safe coupling reagent for amide formation. Although a typical protocol for the use of these reagents involves 2 equiv. of base, usually DIEA, the presence of the morpholinium moiety also allows good results with COMU when only 1 equiv. of DIEA is used. Alternatively, the less basic TMP (2 or even 1 equiv.) can be used instead of DIEA and provides good yields with reduced racemization. Furthermore, COMU is compatible with heat-assisted peptide synthesizers. COMU displays higher efficiency than HATU/HBTU in the demanding synthesis of the Aib derivative of the Leu-enkephalin pentapeptide and produces no Oxyma-based byproduct. Thus, the combination of elevated temperature and COMU results in a similar
Chemical Name:	(1-Cyano-2-ethoxy-2-oxoethylidenaminooxy)dimethylamino-morpholino-carbenium hexafluorophosphate	
CAS:	1075198-30-9	
Molecular structure:		

		performance to that observed by manual synthesis in considerably shorter time.[3]
Trade Name:	TOTU	**TOTU** was described as a peptide coupling reagent in the early 1990s, mimicking the structure of benzotriazolium oxide based uroniumsalts. While uronium salts contain a markedly reactive carbocation core, these moieties stand out as the preferred choice when powerful activation is required. Moreover, the highly polar nature of OxymaPure influences the water solubility of its derivatives, which is essential to removing the coupling byproducts in solution-based acylations. One of the most remarkable advantages over benzotriazole-based reagents is the enhanced solubility of Oxyma-derived byproducts, which results in the preparation of more concentrated coupling mixtures and easier byproduct removal during workup.[4]
Chemical Name:	O-[(Ethoxycarbonyl)cyanomethylenamino]-N,N,N',N'-tetramethyluronium tetrafluoroborate	
CAS:	136849-72-4	
Molecular structure:		
Trade Name:	K-Oxyma	**K-Oxyma**, the potassium salt of OxymaPure, shows impressive and outstanding benefits. A major benefit is the complete suppression of the acidity of OxymaPure making K-Oxyma the most suitable reagent choice when peptides are assembled in highly acid-labile solid-supports, as it avoids premature release of the peptide from the resin. K-Oxyma has a greater solubility than OxymaPure.[5]
Chemical Name:	2-Cyano-2-(hydroxyimino)acetic acid ethyl ester, potassium salt, Ethyl (hydroxyimino)cyanoacetate potassium salt	
CAS:	158014-03-0	
Molecular structure:		
Trade Name:	Oxyma-B	In order to overcome the high reactivity of OxymaPure, we have synthesized Oxyma-B as a new oxime derivative. Research results indicate that **Oxyma-B** is a superior racemization suppressor compared to OxymaPure and HOAt (which to date was considered the most outstanding). In addition, the presence of the carbonyl groups oriented in the same direction as the N-OH group in this
Chemical Name:	5-(hyd5-(hydroxyimino)-1,3-dimethylpyrimidine-2,4,6(1H,3H,5H)-trioneroxyimino)-1,3-dimethylpyrimidine-2,4,6(1H,3H,5H)-trione	
CAS:	5417-13-0	

Molecular structure:		molecule, increase the reactivity of Oxyma-B. Hence, these groups enhanced basic catalysis; thereby improving the nucleophilicity of the amine function during coupling. Importantly, Oxyma-B does not contain any ethyl ester, which can lead to side reactions.[6]
Trade Name: **Chemical Name:** **CAS:** **Molecular structure:**	Fmoc-AmOX Acetamide, 2-cyano-2-[[[(9*H*-fluoren-9-ylmethoxy)carbonyl] oxy]imino]- 1370440-28-0 	In the search for the ultimate active species capable of providing optimal Fmoc-introduction, **Fmoc-AmOX** was developed. Fmoc-AmOX provides high stability and impressive reactivity leading to less than 0.1% content of undesired dipeptides and undesired enantiomers.[7]
Trade Name: **Chemical Name:** **CAS:** **Molecular structure:**	Py-Oxim [Ethyl cyano(hydroxyimino)acetato-O2]tri-1-pyrrolidinylphosphonium hexafluorophosphate 153433-21-7 	**PyOxim** shows higher stability in acetone and DMF than all benzotriazole counterparts, consequently standing out as a promising choice for peptide cyclization.[8]

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