T-BEC® carbodiimide and OxymaPure® for Sustainable SPPS

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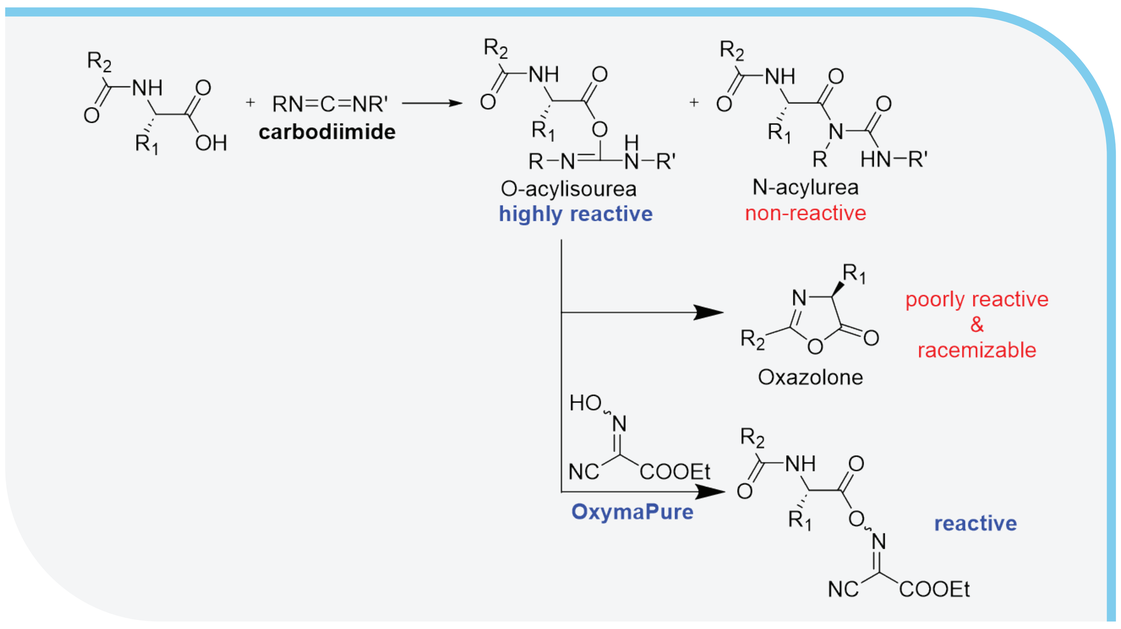
Introduction:

Carbodiimides (CDI) are essential compounds in peptide and amide synthesis, facilitating the conversion of carboxylic acids into amides or esters. This process can lead to unwanted byproducts such as N-acylurea or oxazolone due to the rearrangement of O-acylisourea. To improve yields and reduce side reactions, CDIs are often combined with additives like hydroxybenzotriazoles or OxymaPure®, which is safer and more effective alternatives to benzotriazole (Scheme 1). In peptide synthesis, three common CDIs are DCC, DIC, and EDC-HCl. DCC is avoided in solid-phase peptide synthesis (SPPS) due to its toxicity and low solubility of the urea byproduct. EDC-HCl, while water-soluble, has a high racemization rate.

Currently, DIC combined with OxymaPure® is widely used in Fmoc/tBu-AA SPPS in DMF/NMP solvents. However, this approach has significant drawbacks.

- During Fmoc-AA pre-activation, DIC can react with OxymaPure®, producing oxadiazole and hydrogen cyanide (HCN) as byproducts (Scheme 2).
(DOI: 10.1021/acs.oprd.9b00344).

- The removal of diisopropylurea (DIU), a byproduct of DIC-mediated couplings, is challenging due to its low solubility, leading to equipment clogging during operations.
(DOI: 10.1039/d0ra07204d)



Scheme 1: Pathways of Carboxylic Acid Activation by Carbodiimides and Racemization Suppressors

Objective:

The objective of this research is to enhance the efficiency and safety of peptide synthesis by evaluating different carbodiimide (CDI) reagents and their combination with OxymaPure® as alternatives to the commonly used DIC in peptide synthesis. This addresses issues such as the formation of hydrogen cyanide (HCN) and the low solubility of diisopropylurea (DIU) byproducts. The aim is to provide a more efficient and safer approach to peptide synthesis, benefiting both the scientific community and the industry.

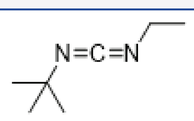
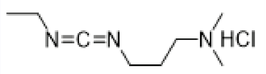
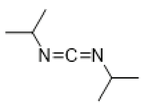
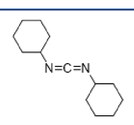
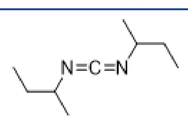
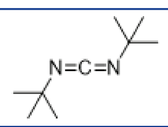
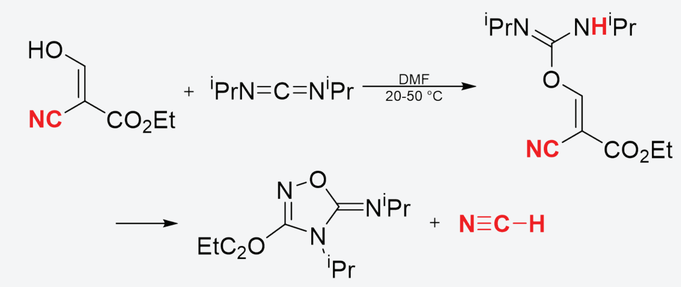
CDI	Formula	additive	Oxadiazine	Oxadiazole + HCN	Amide Bond formation
T-BEC		OxymaPure®	**Yes**	No	+++
EDC-HCl			**Yes**	No	++
DIC			**Yes**	**Yes**	+++
DCC			No	**Yes**	+++
DSBC			No	**Yes**	+
DTBC			**Yes**	No	-

Table 1: Reaction of various CDIs with OxymaPure®

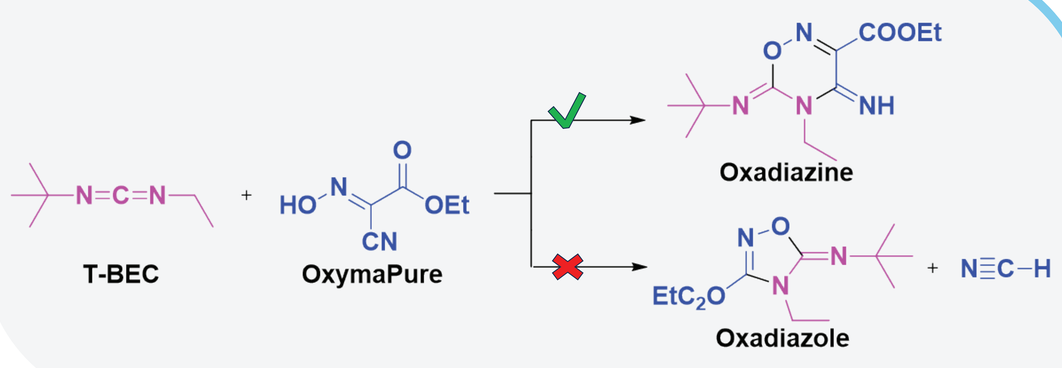


Scheme 2: Formation of Oxadiazole and Hydrogen Cyanide (HCN) during Preactivation with DIC and OxymaPure.

Formation of HCN was indirectly detected through LC-HRMS analysis, which revealed that DIC reacts with OxymaPure® at a 1:1 ratio, predominantly yielding Oxadiazole and HCN. In contrast, T-BEC® reacts with OxymaPure® under the same conditions, forming Oxadiazine (Table 1, Scheme 3).
(DOI: 10.1021/acs.orglett.1c02466)

	DIC / OxymaPure®	T-BEC®/ OxymaPure®
	Purities of the Peptides (HPLC, %)	
Leu-enkephalin	99.9%	96.7%
Ile2,3-Leu-enkephalin	98.4%	96.6%
65-74 ACP	89.49%	96.12%
ABRF92	73.78%	74.53%
H-A10K3-NH2	85.30%	87.93%
	Racemization study (DL/LL, %)	
H-Gly-His-Phe-NH2	1.58%	1.05%
H-Gly-Cys-Phe-NH2	N.D	N.D
H-Gly-Ser-Phe-NH2	0.27	0.29

Table 2: Comparison of the Effects of DIC/OxymaPure® and T-BEC®/OxymaPure® on the Purity of Long and Short Peptides, Along with a Racemization Study.



Scheme 3: Proposed Mechanism of the Reaction of T-BEC® with OxymaPure®.

Solid-phase peptide synthesis (SPPS) on Rink amide resin, using 5 equivalents of reagents, in situ activation in DMF at 25°C for 30 minutes (Table 3).

The solubility study of urea in various solvents and binary solvent systems revealed that TBEU is significantly more soluble than DIU.
(DOI: 10.1021/acs.oprd.3c00120)

Conclusions:

T-BEC® has emerged as an efficient carbodiimide that prevents the formation of HCN, and its byproduct urea (TBEU) is soluble in various conventional, green, and binary solvents. Therefore, T-BEC® presents itself as an ideal candidate for replacing DIC in green SPPS chemistry, offering improved safety and performance in peptide synthesis.

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