TBDC® and QCD®: Novel water soluble carbodiimides for TAG assisted LPPS and minimal protection synthesis

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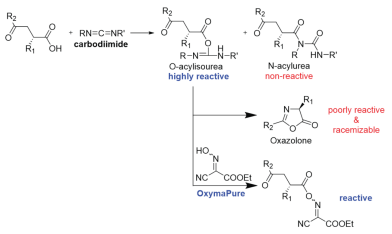
Introduction:

Carbodiimides (CDIs) are essential compounds in amide and peptide synthesis, enabling the activation of carboxylic acids for coupling with amines. However, the O-acylisourea intermediates they generate are unstable and prone to side reactions, such as rearrangement into N-acylureas or oxazolones, leading to product loss and impurity formation.

To minimize these issues, CDIs are routinely paired with nucleophilic additives- generally safe and efficient OxymaPure® is used, which reduces racemization and improves coupling efficiency.



Water-soluble carbodiimides (WSC) are used in liquid-phase synthesis (LPPS). They are ideal candidate for aqueous-based reactions and bioconjugation, and non-aqueous media for peptide synthesis as generating a water-soluble urea byproduct, making it easy to separate from the crude product.

Today, the only water soluble carbodiimide EDC-HCl dominates in the peptide industry. This is the less effective CDI that exhibit high racemization risk, reduced stability and high toxicity. In addition, EDC HCL has limited solubility in green and binary solvents.

Recently, TAG-assisted LPPS technologies have shown an increasing demand for novel and more effective WSC, compatible with green and binary solvents enabling straightforward removal of coupling byproducts by aqueous extraction without compromising reaction efficiency or product purity.

Innovation at a Glance

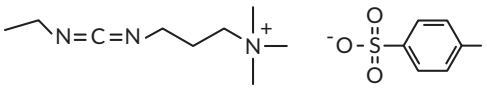
We introduce TBDC® and QCD®—two next-generation water soluble CDIs designed to overcome EDC HCl limitations while maintaining high reactivity, safer profiles, and increased solubility and broad compatibility with both SPPS and LPPS protocols including TAG assisted LPPS. In addition, TBDC® allows to use side chain unprotected AA without compromising peptide purity.

These reagents combine synthetic efficiency with green chemistry principles, aligning with the latest regulatory and environmental standards.

TBDC® reagents are patent-pending products by Luxembourg Bio Technologies Ltd.

QCD®

Designed for use in LPPS and SPPS. More stable, more efficient than EDC-HCL. Green CDI that employs the same coupling protocols as other carbodiimides.



Racemization:
QCDs exhibited similar or slightly better racemization profiles compared to EDC.HCl.

Efficiency:
Improved performance in SPPS, yielding higher purity peptides.

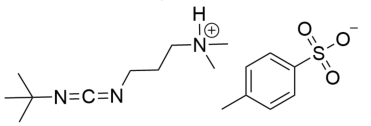
	EDC-HCl/ OxymaPure® (Purity %)	QCD® / OxymaPure® (Purity %)
Leu-enkephalin (H-Tyr-Gly-Gly-Phe-Leu-NH)	90	95.3
Ile2,3-Leu-enkephalin (H-Tyr-Ile-Ile-Phe-Leu-NH2)	81.7	97.5

Safety:
Preliminary tests indicate that QCDs are safer, with reduced toxicity concerns.

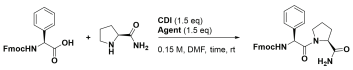
Reagent	EDC.HCl	QCD®
Hazard profile	Acute tox. (Cat.3)	LD50 > 10,000 mg/kg
	Acute tox (Cat. 4)	Not a skin irritant
	Irritation (Cat.2)	Not an eye irritant
	Sens. (Cat.1)	
	(STOT RE 2)	
	Environmental Hazards	
	Acute aquatic toxicity (Cat.1)	
	Chronic aquatic toxicity (Cat.1) Acute toxicity LD50 oral- 500 mg/kg	

TBDC® HCl & TsOH:

Water soluble carbodiimide for minimal protection LPPS and TAG assisted LPPS.

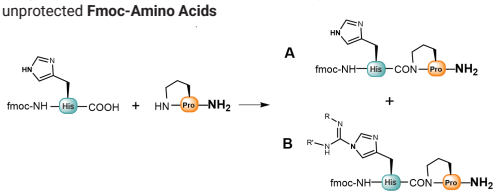


CDIs efficiency in the condensation between Fmoc-L-phenyglycine and L-prolinamide



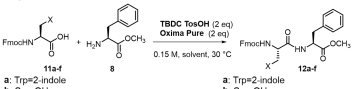
CDI	Additive	C_{max} % HPLC	Time (min)	L/D[b]
EDC HCl	OxymaPure	100%	15min	93:7
TBDC HCl	OxymaPure	100%	30min	97:3
TBDC TsOH	OxymaPure	100%	30min	97:3
EDC HCl	HOBt	100%	15min	75:25
TBDC HCl	HOBt	100%	15min	81:19
TBDC HCl	HOAt	100%	15min	87:13
TBDC HCl	Oxyma-B	100%	60 min	96:4

Coupling do not result in side reactions, allows you to use side chain unprotected **Fmoc-Amino Acids**



Carbodiimide	Conversion %	A/B
EDC · HCl	>99	75/25
TBDC · HCl	>99	>99/1
TBDC · TsOH	>99	>99/1

Screening of coupling between amino acids unprotected on the side-chain and H-Phe-OMe

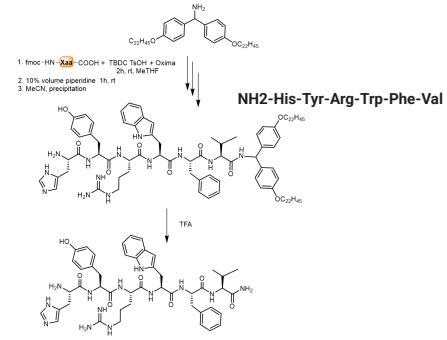


a: Trp=2-indole
b: Ser=OH
c: Thr=CH(OH)CH_3
d: Tyr=4-OH-Ph
e: Arg=$CH_2CH_2C(=NH)NH_2$ HCl
f: His =2-Imidazole

Entry	AA	Solvent	C_{max} %	Time (h)
1	Fmoc-Trp-OH	DMF	100	1h
2		MeTHF	100	1.5h
3	Fmoc-Ser-OH	DMF	100	1h
4		MeTHF	100	1.5h
5	Fmoc-Thr-OH	DMF	100	1h
6		MeTHF	100	0.5h
7	Fmoc-Tyr-OH	DMF	100	0.5h
8		MeTHF	100	0.5h
9	Fmoc-Arg-OH	DMF	100	0.5h
10		MeTHF	100	0.5h
11	Fmoc-His-OH	DMF	100	2 h
12		MeTHF/DMSO 9/1	100	3 h

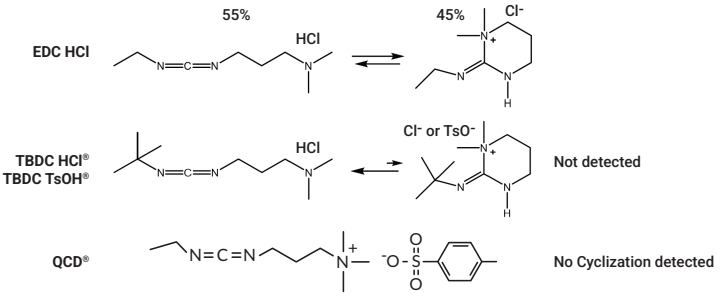
a. 4 equivalents of Oxima Pure have been added.
b. The addition of 10% DMSO was necessary to overcome the scarce solubility of fmoc-His-OH in MeTHF and 3 equivalents of Oxima Pure.

TAG assisted synthesis



The final product was obtained in >99% purity and >80% yield after treatment with TFA and precipitation with isopropyl ether.

Stability: the open form of EDC.HCl is in a tautomer equilibrium with a six-member ring form, which is ineffective.
The resence of the quaternary N in QCD block the tautomerism possibility, while hindered tertbutyl prevents cyclization of TBDC®.



Solubility: QCD® and TBDC® are soluble in both water and organic green and binary solvents, enhancing their versatility.

Solvent	EDC·HCl	TBDC·HCl	TBDC·TsOH	QCD
DMF				
DCM				
Anisole				
DMC				
DEC				
NOP				
NBP				
NBP/DMC 8/2				
EtOAc				
DMSO				
EtOAc/DMSO 7/3				
MeCN				
MeCN/DMSO 7/3				
PrOAc				
PrOAc				
BuOAc				
CPME				
2-MeTHF				
2-MeTHF/DMSO 9/1				
GVL				
DOS				
TEP				
Water				

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