

PyClock[®], A New Phosphonium Salt from 6-Chlorohydroxybenzotriazole (Cl-HOBt) for Peptide Coupling

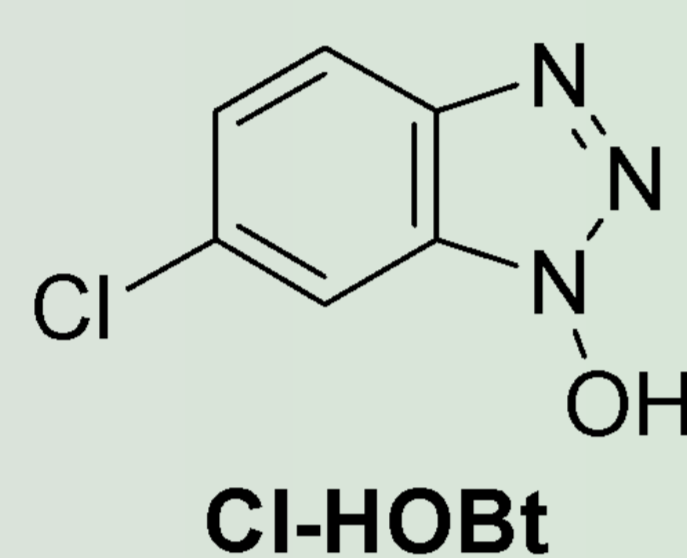
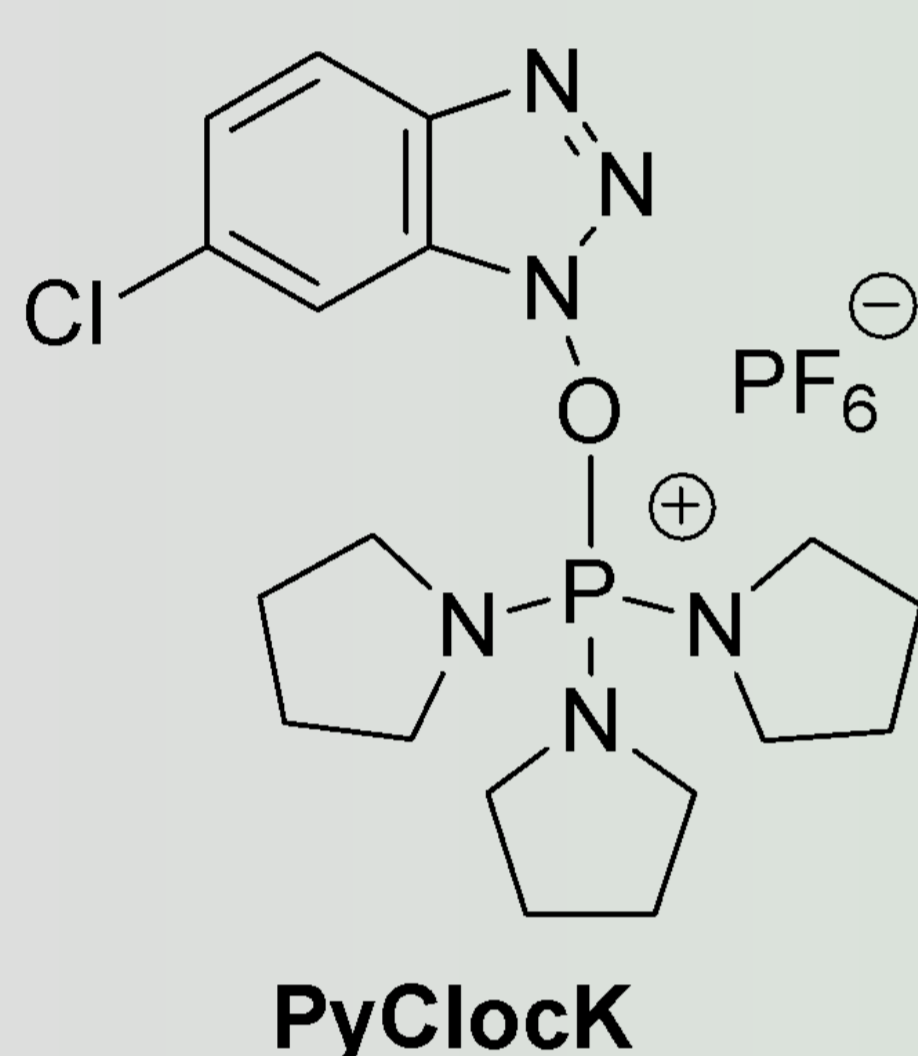
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INTRODUCTION

The peptide coupling reagent field has clearly evolved in the last decade from carbodiimides to onium (phosphonium and uronium) salts. The era of industrial coupling reagents began in 1955 with the introduction of dicyclohexylcarbodiimide (DCC), which at that time was already known and well studied, as a reagent for the formation of amide bond. Unfortunately, carbodiimides did not comply with the concept of ultimate coupling reagents because its high reactivity provokes racemization and side reactions during the coupling reaction.



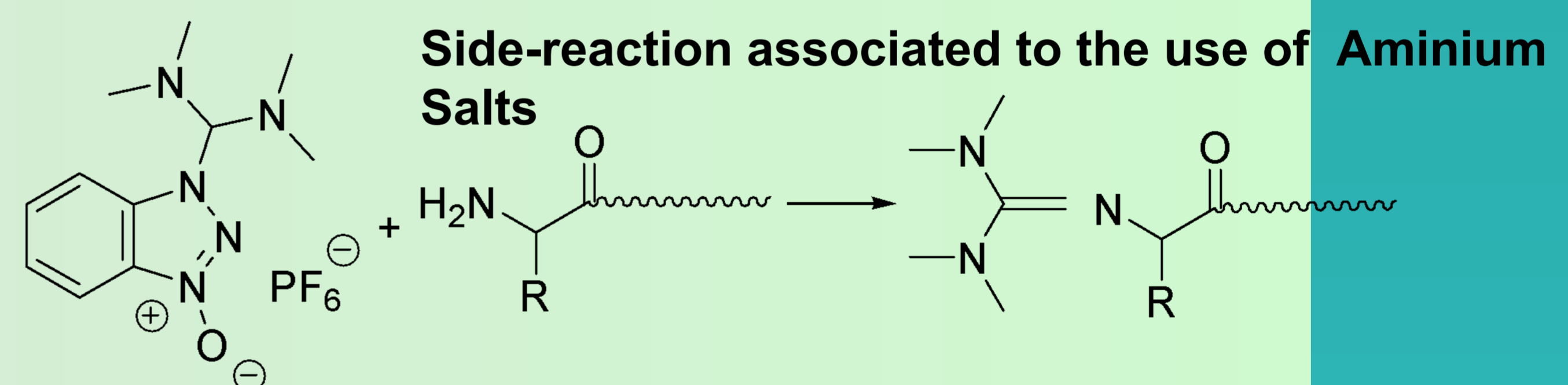
At the beginning of the 70's, 1-hydroxybenzotriazole (HOBt) was proposed as an additive to DCC to reduce racemization and from then on other benzotriazole derivatives such as 1-hydroxy-6-chlorobenzotriazole (Cl-HOBt) or 1-hydroxy-7-azabenzotriazole (HOAt) have also been used. The OBt active esters are less reactive than the O-acylisourea, but are more stable and less prone to racemize.

The use of the most reactive aminium salt, HATU, is inconvenient because of the price, which makes its use detrimental for industry. HCTU/TCTU, based on Cl-HOBt, are a good alternative to HBTU/TBTU, because of the presence of the chlorine atom that stabilizes the structure, hence, making these reagents less hazardous.

Herein, PyClock, the phosphonium salt of the Cl-HOBt is introduced.

ADVANTAGE OF THE PHOSPHONIUM SALTS IN FRONT OF THE AMINIUM SALTS

PyClock as other phosphonium salts can not react with the free amine function and therefore does not terminate the peptide growing as the aminium salts do, through the formation of guanidine derivatives. Thus, **PyClock** is specially useful for slow coupling reactions and it can be used in excess to assure a complete activation of the carboxylic function.



REACTIVITY OF THE PHOSPHONIUM SALTS IN FRONT OF THE AMINIUM SALTS

Stability of these kind of reagents can be used as a measure of their reactivity. The results in the Table shows that PyAOP, derived from HOAt, is the most unstable indicating its higher reactivity. Furthermore, **PyClock** shows more stability than PyAOP, but more unstable than PyBOP. Thus, **PyClock** is more reactive than PyBOP.

	PyClock		PyBOP		PyOAP	
	6-Cl-HOBt	PyClock	HOBt	PyBOP	HOAt	PyOAP
t = 6h	31.18	68.82	22.15	77.85	34.74	65.26
t = 9h	49.04	50.96	32.13	67.87	53.06	46.94
t = 24h 20min	99.96	0.04	98.91	1.09	99.84	0.16

ACTIVATION OF THE HINDERED AMINO ACID, Aib

PyClock is faster than PyBOP in activating hindered amino acids such as Fmoc-Aib-OH

	PyClock (% HPLC)		PyBOP (% HPLC)	
	Fmoc-Aib-OH	Fmoc-Aib-OCi-OBt	Fmoc-Aib-OH	Fmoc-Aib-Obt
t = 2 min	18.08	81.92	32.51	67.49
t = 1h	14.13	85.87	31.45	68.55
t = 2h	21.38	78.62	27.27	72.73

PyClock[®] is a proprietary product of Luxembourg Industries Ltd.

SYNTHESIS OF HINDERED DERIVATIVES OF LEU-ENKEPHALINAMIDE

- H-Tyr-Aib-Aib-Phe-Leu-NH₂

Aib	Purity (% HPLC)		
	PyClock	PyBOP	PyAOP
H-Tyr-Aib-Aib-Phe-Leu-NH ₂	97.24	85.11	99.47
Impurity: H-Tyr-Aib-Phe-Leu-NH ₂	2.76	14.89	0.53

- H-Tyr-Arg-Arg-Phe-Leu-NH₂

Arg(Pbf)	Purity (% HPLC)	
	PyClock	PyBOP
H-Tyr-Arg-Arg-Phe-Leu-NH ₂	91	82
Impurity: H-Tyr-Arg-Phe-Leu-NH ₂	9	18

In both cases, **PyClock** shows a clear superiority in front of PyBOP

CONCLUSIONS

Results presented in this work indicate that **PyClock** is superior to PyBOP as coupling reagent. Furthermore, in some cases its reactivity is close to the most active reagent, PyAOP.