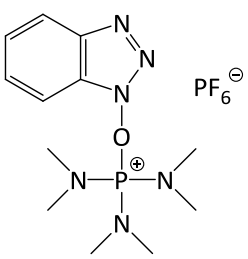
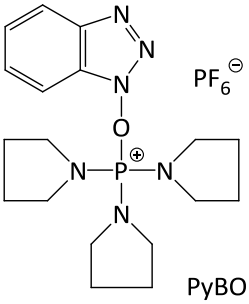
Coupling Reagents

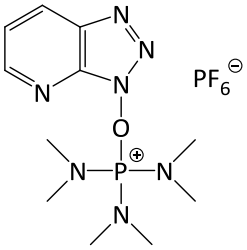
1. COUPLING REAGENTS : Structure and acronyms



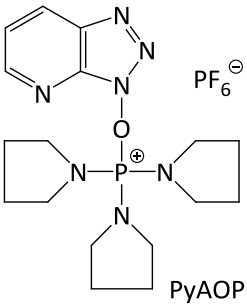
BOP



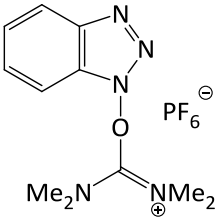
PyBOP



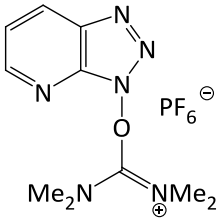
AOP



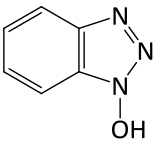
PyAOP



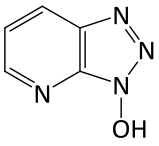
HBTU



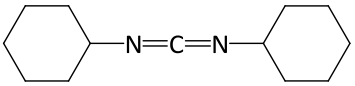
HATU



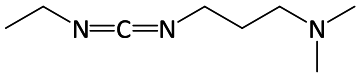
HOBt



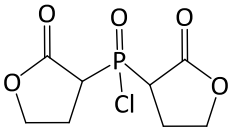
HOAt



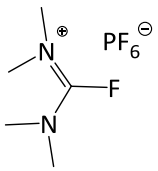
DCC



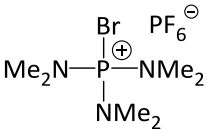
EDCI



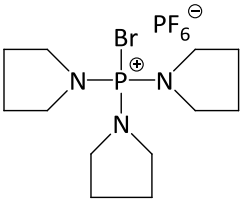
BOP-Cl



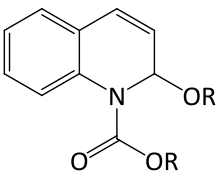
TFFH



Brop

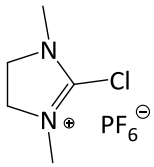


PyBrop

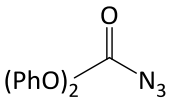


EEDQ : R = Et

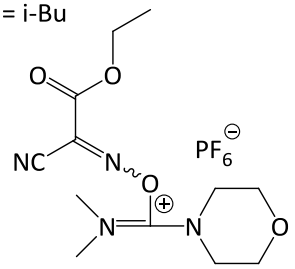
IIDQ : R = i-Bu



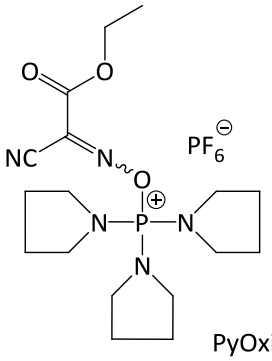
CIP



DPPA



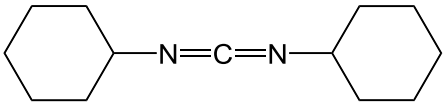
COMU



PyOxim

2. CARBODIIMIDE

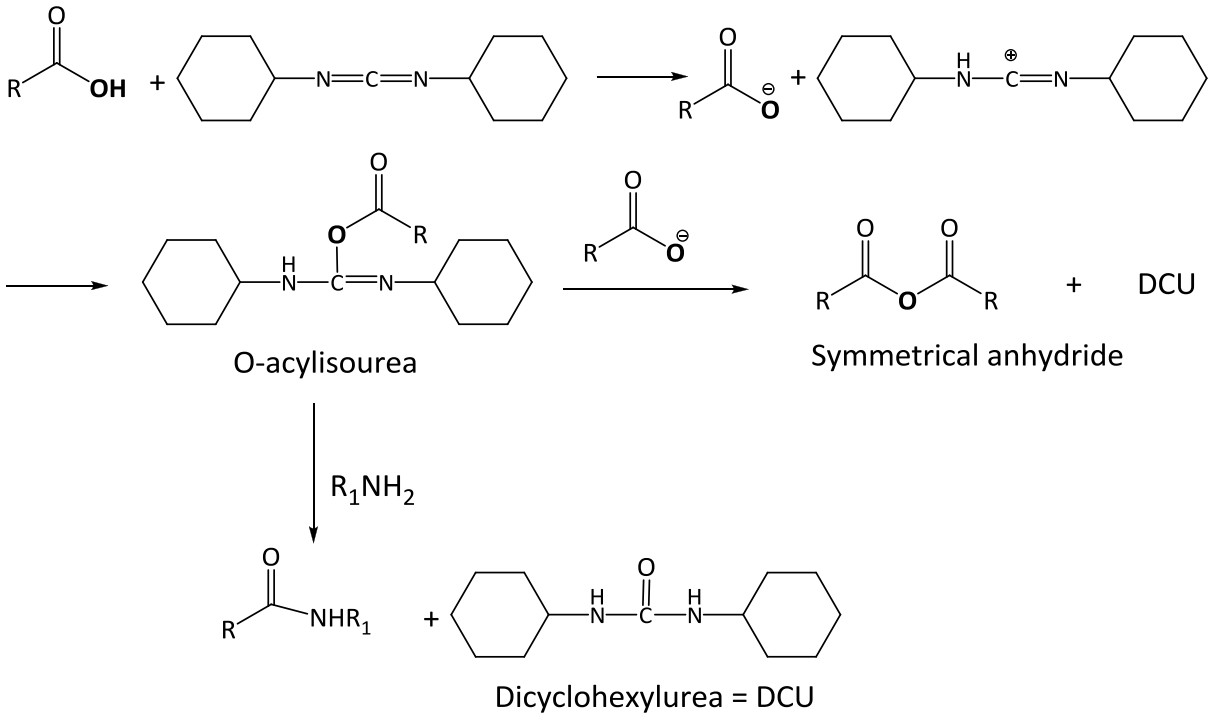
DCC and EDC are often used with an additive: This is probably the most common method of coupling segments, with HOBt as the most efficient additives. The additive is essential to reduce isomerization to acceptable levels.



1.a. N,N'-Dicyclohexylcarbodimide (DCC)

Introduced in peptide synthesis by Sheehan and Hess in 1955. [1]

Mechanism



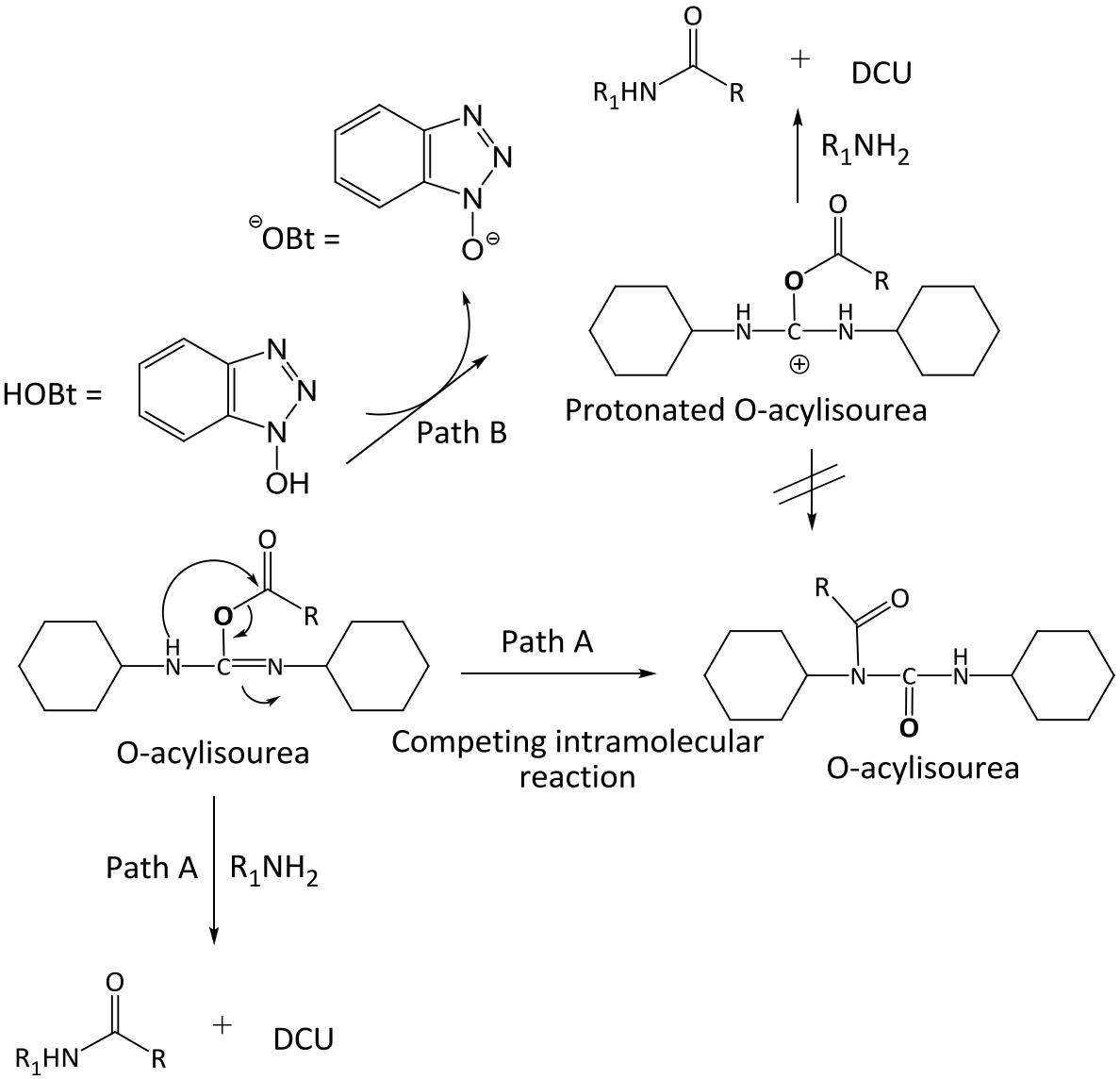
Symmetrical anhydrides are stable enough to be isolated but not stable enough to be stored for future use.

Problem

when DCC is used alone (transposition O to N acylurea is observed Path A)

[1] JC Sheehan, GP Hess. A new method of forming peptide bonds. (carbodiimide) *J. Am. Chem. Soc.* **1955**, *77,* 1067.

Side product observed when DCC is used alone (transposition O to N acylurea Path A)



1-Hydroxybenzotriazole (HOBt) as an additive that suppresses *N*-acylurea formationby protonation of the *O*-acylisourea (Path B).[2]

DCC/HOBt coupling experimental procedure:

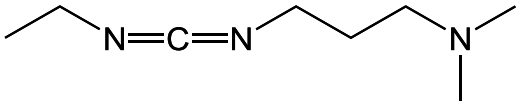
Synthesis of N-CBZ-Gly-L-Phet-butyl ester dipeptide

1. To a mixture of N-CBZ-Gly (837 mg, 4.0 mmol) and HOBt (536 mg, 4 0 mmol) in 30 mL of ethyl acetate chilled in an ice-water bath, add DCC (906 mg, 4.4 mmol) in one portion. Stir the mixture for 30 mm, and remove the ice bath. Formation of white precipitate (DCU) is observed.
2. Add L-Phe-OtBu (103 1 mg, 4.0 mmol) and TEA (556 µL, 4 0 mmol). Continue stirring the mixture at RT for 2 h. Pour 15 mL of hexanes into the mixture with shaking.
3. Store the mixture in a freezer overnight. Remove DCU by filtration Wash the precipitates on a fritted funnel with 10 mL of ethyl acetate

[2] DH Rich, J Singh. The carbodiimide method, in E Gross, J Meienhofer, eds. *The Peptides: Analysis, Synthesis, Biology*, Academic, New York, **1979**, Vol 1, pp 241-261.

4. Wash the combined filtrates with 4% HCl(30 mL x 2), saturated $NaHCO_3$ solution (25 mL x 3) and brine (30 mL x 3), and dry the mixture over anhydrous $MgSO_4$. Filtration and solvent evaporation give 1.55 g (94%) of dipeptide.

1.b. N-(3-Dimethylaminopropyl)-*N'*-ethylcarbonate (EDC) [3]



Water Soluble by-product is easily removed in aqueous work-up
EDC with an additive: This is probably the most common method of coupling segments, with HOBt as the most efficient additives.
Additive = HOBt

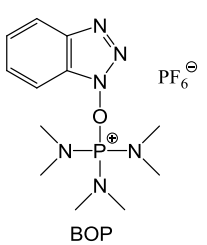
EDC coupling experimental procedure:

EDC,HCl (1.20 equiv) and **N, N- diisopropylethylamine** (2.5 equiv.) were added to a solution of **amine** (1 equiv.), **acid** (1 equiv.) and **HOBt** (1.2 equiv.) in **DMF** (10ml/1mmol) and stirred for 24 hours at room temperature under nitrogen. The solvent was removed in vacuo and the residue purified by column chromatography on silica gel

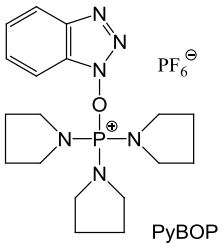
3. Phospohnium & uroniums

2.a (benzotriazol-1-yl-oxy-*tris*(dimethylamino) phosphonium hexafluorophosphate) BOP [4], and PyBOP

BOP first use in 1975. Reagents bearing the dimethyl amine moiety produce HMPA as a toxic byproduct, and thus their pyrrolidine based analogues are preferred.



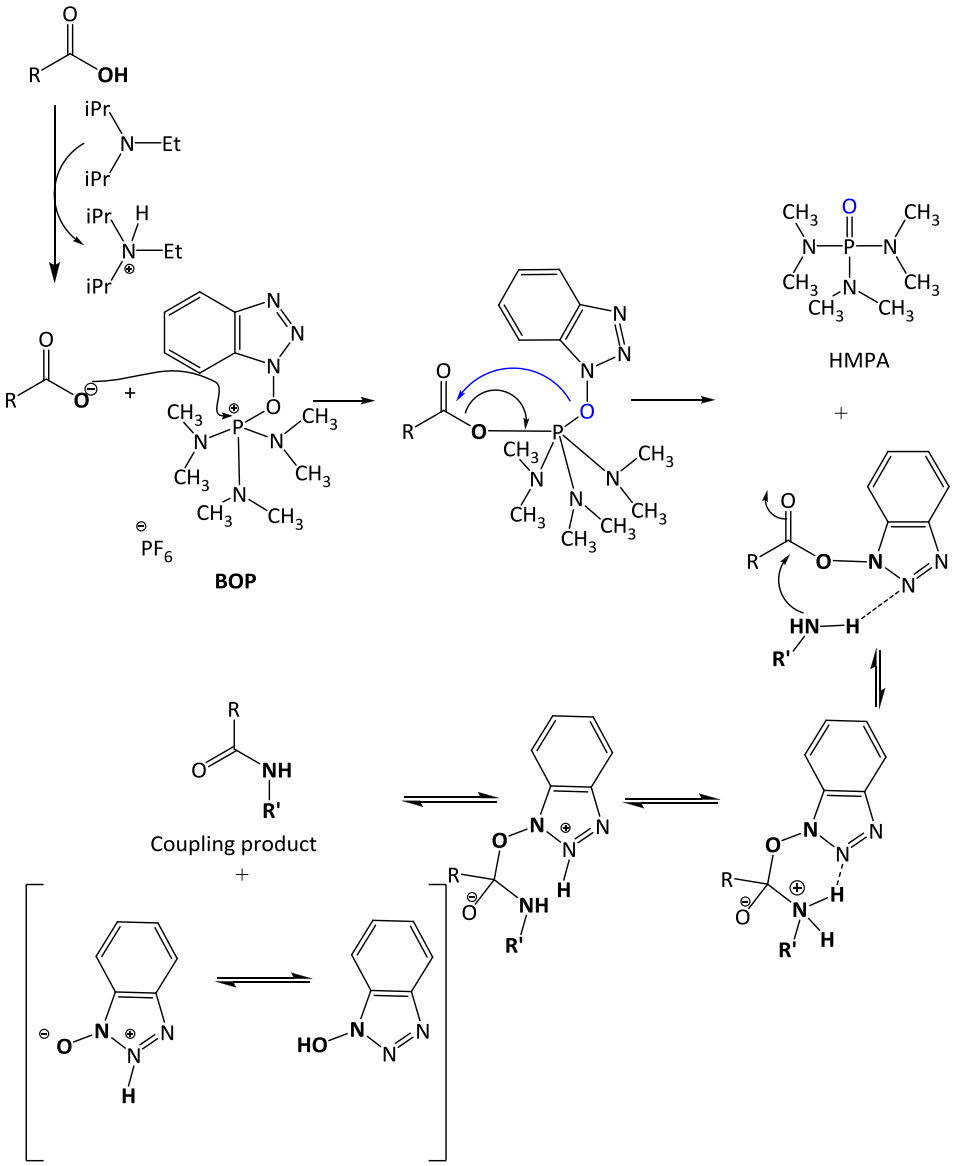
BOP



PyBOP

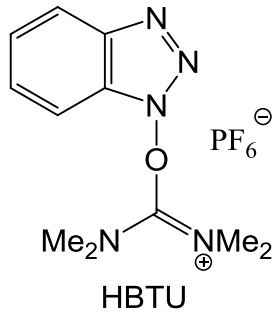
[3] JC Sheehan, PA Cruickshank, GL Boshart. Convenient synthesis of water-soluble carbodiimides. *J. Org. Chem.* **1961**, *26*, 2525.

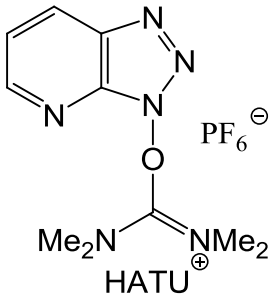
[4] B Castro, JR Dormoy, G Evin, B Castro. Peptide coupling reagents IV (1) benzotriazole *N*-oxytrisdimethylamino phosphonium hexafluorophosphate (B.O.P.) *Tetrahedron Lett* **1975**. 1219.



2.b. (*O*-benzotriazol-1-yl-*N*,*N*,*N*2,*N*2-tetramethyluronium hexafluorophosphate) HBTU [5]

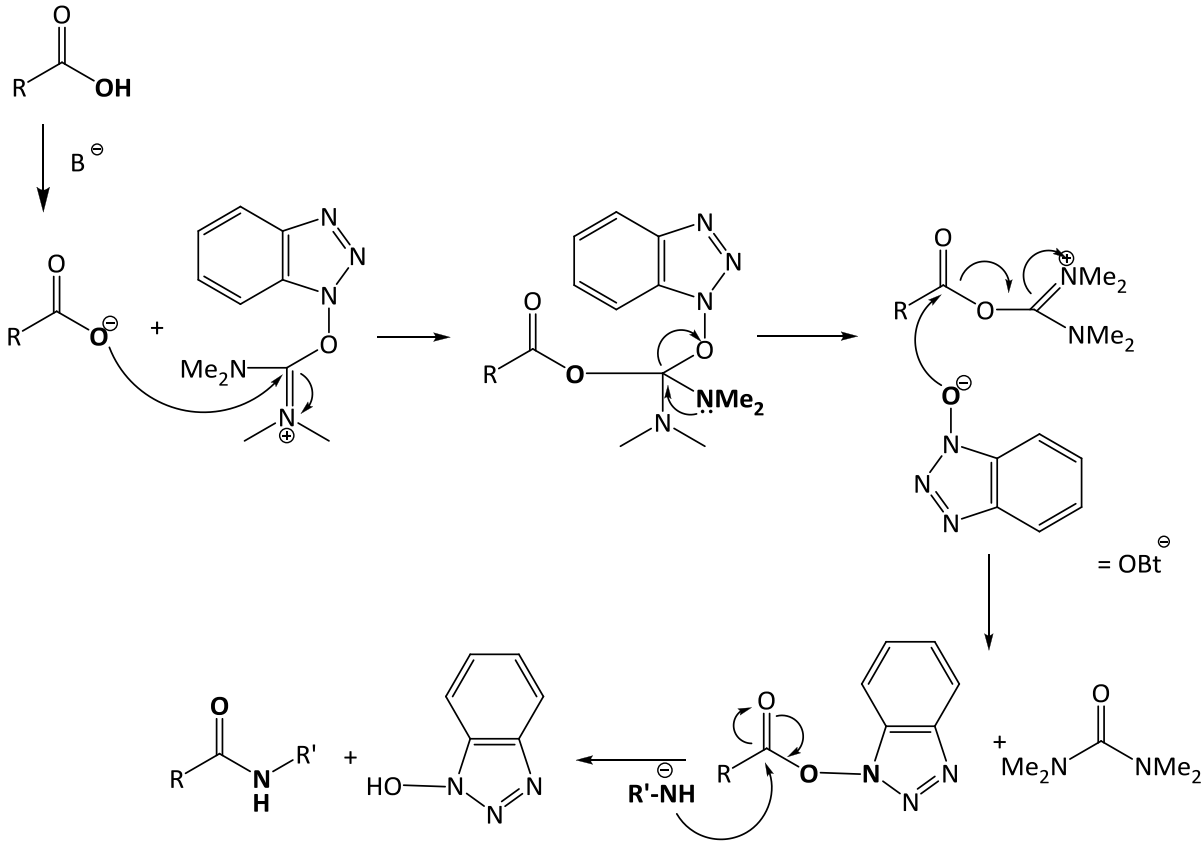
HBTU first use in 1978





[5] V Dourtoglou, J-C Ziegler, B Gross. L'hexafluorophosphate de *O*-benzotriazolyl-*N*,*N*tetramethyluronium hexafluorophosphate: a new and efficient peptide coupling reagent. Tetrahedron Lett. **1978**, 1269.

Mechanism of HBTU

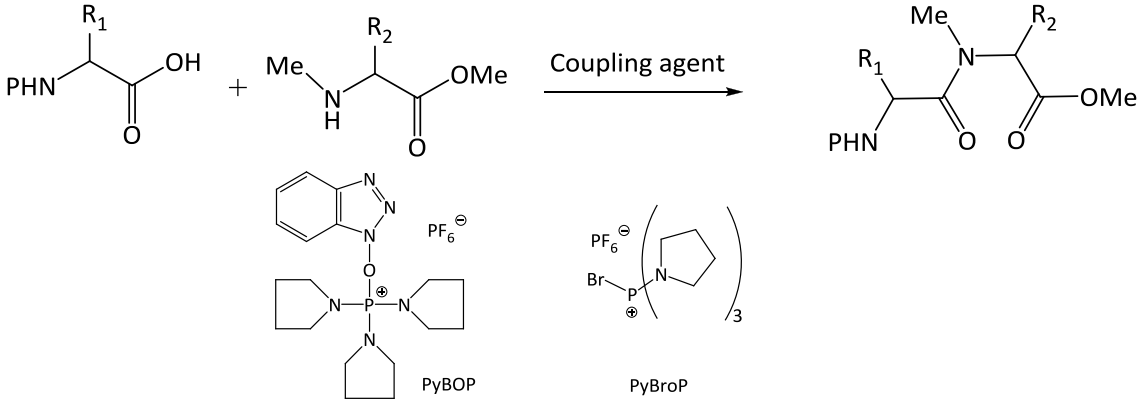


HBTU coupling experimental procedure:

To a suspension of **acid** (1equiv.), **amine** (1.1 equiv.) and **Et_3N** (3.5 equiv.) in **DMSO** (30 mL/ 10 mmol) was added **HBTU** (3.5 equiv.) and the mixture stirred at room temperature for 18 hours. The reaction was then diluted with EtOAc (100 ml) before washing with water (100 mL) and brine (3 x 100 mL). The EtOAc layer was dried (Na_2SO_4), filtered and concentrated in vacuo.

4. Secondary amine coupling

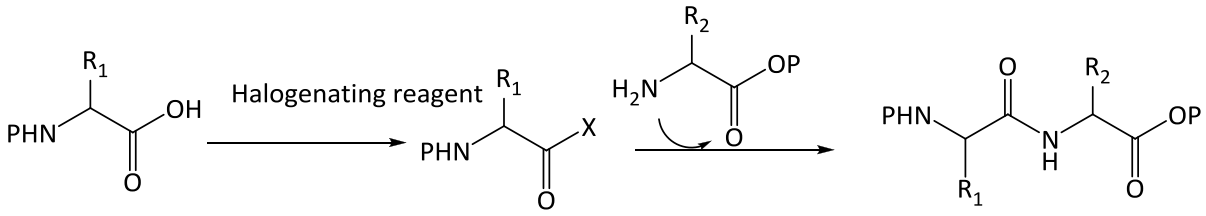
More powerful activation is needed for secondary amines : [6]



Sequence	% Yield (hours)	
	PyBOP	PyBroP
Z-**Me**Val-Val-OMe	90 (1)	85 (1)
Z-Val-**Me**Val-OMe	11 (1)	70 (1)
Fmoc-Val-**Me**Val-OMe	30 (24)	84 (3)
Boc-Val-**Me**Val-OMe	45 (24)	44 (3)

CH_2Cl_2, DIEA, room temp.

5. Acid halogenations



X = Cl or F

Common reagents to make acid chlorides
Oxalyl chloride
Thionyl chloride ($SOCl_2$)
Phosphorous oxychloride ($POCl_3$)

Currently there are a number of commercially available Fmoc amino acid fluorides, however Boc and Cbz groups present problems in the coupling of acid halides.

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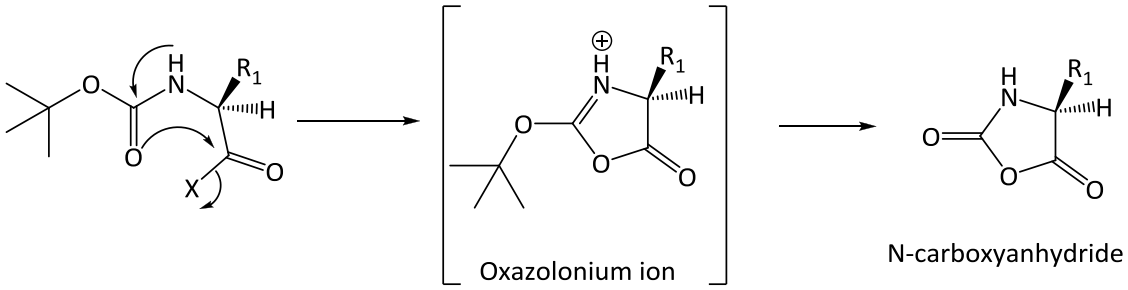
[6] Coste, J.; Frerot, E.; Jouin, P. Coupling N-Methylated Amino Acids Using PyBroP and PyCloP Halogenophosphonium Salts: Mechanism and Fields of Application. *J. Org. Chem.* **1994**, *59*, 2437-2446.

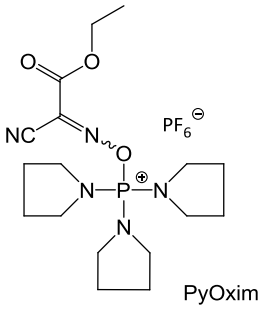
Carboxyanhydride Formation: An Unwanted Side reaction



This side product can be significantly reduced with careful selection of protecting groups for the amine functionality (use of sulfonyl protecting group).

6. Recent coupling reagents

6.a (Ethyl cyano(hydroxyimino)acetato-O2)-tri-(1-pyrrolidinyl)-phosphonium hexafluorophosphate. (PyOxim)[7]

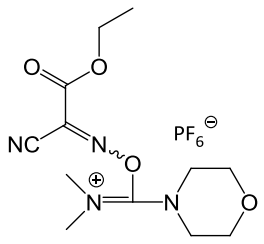


PyOxim is a novel reagent which mediates coupling reactions with efficiencies superior to HATU, TBTU, PyBOP and comparable to COMU. It is has excellent solubility in DMF and is stable in solution under an inert atmosphere for two days. Unlike HATU and HBTU,

- it cannot cause chain terminating side reactions and is, therefore,
- ideal for the synthesis of cyclic peptides.
- Furthermore, it is not explosive under normal operating conditions
- and is less likely to exhibit allergenicity compared to other coupling reagents.

[7] Subiros-Funosas, R. et al. *Org. Biomol. Chem.* **2010**, 8, 3665.

6.b. 1-[(1-(Cyano-2-ethoxy-2-oxoethylideneaminooxy)-dimethylamino-morpholino)] uranium hexafluorophosphate (COMU)[8]



COMU

ethyl (hydroxyimino)cyanoacetate are used to substitute the benzotriazole moiety as a leaving group

- **COMU**, are not explosive under normal operating conditions
- and, importantly, allergic reactions such as contact dermatitis or asthma are drastically reduced or eliminated.

[8] "COMU: A Safer and More Effective Replacement for Benzotriazole-Based Uronium Coupling Reagents", El-Faham, et al. *Chem. Eur. J*. **2009**, *15*, 9404.