Iris Biotech

COUPLING REAGENTS

Version: IB12_1

Empowering Peptide Innovation

With this guiding theme in mind, Iris Biotech's mission is to support researchers by supplying

- innovative technologies,
- rare compounds,
- as well as a broad portfolio on standard consumables,

available in flexible quantities from small scale to bulk quantities. To fulfill our dedication "Empowering Peptide Innovation", we are attending various conferences, symposia, and exhibitions each year. This allows us to remain in direct contact with scientists all over the world, both from academia and industry, to exchange knowledge, and to gather new ideas to tackle your current challenges.

Guided by our dedication to provide

- competent service,
- as well as novel substances and
- latest technologies,

Iris Biotech is your trusted partner for the world of peptides, while having strong expertise in associated disciplines. Thus, our portfolio comprises reagents and tools for the synthesis and modification of peptides, e.g., amino acids, resins and solvents but also for related technologies such as drug delivery, linkerology® and life sciences.

Owed to the growing demand for tailor-made compounds, our portfolio is fine-tuned by our custom synthesis service at Iris Biotech Laboratories. Our skilled scientists offer profound expertise in

- *de novo* route development,
- upscaling towards larger scale production,
- as well as synthesis optimization for increased efficiency.

Examples are the synthesis of rare chiral building blocks, unnatural amino acid derivatives, sophisticated orthogonal protecting groups, heterocycles, building blocks for nucleotides, PEGs and PEG-analogs as well as specific linkers for controlled drug delivery and release.

Amino Acids ***Building Blocks*** ***Life Sciences*** ***Drug Delivery*** ***Reagents*** ***Resins*** ***Linkerology®*** ***Click Chemistry***

Portfolio Overview

Peptide Synthesis and Modification

(Protected) Amino Acids

Standards such as Fmoc-D/L-AAA and Boc-D/L-AAA, Smoc amino acids for peptide synthesis in water, variety of protecting groups (e.g., Pbf, Trt, ^{t}Bu, Bzl, Acm, Mob, SIT, Phacm, Allocam, Mmt), unusual amino acids, fluorinated derivatives, substituted prolines, arginine analogs

Building Blocks

Amino alcohols, amino aldehydes, diamines and hydrazines, (pseudoproline) dipeptides, polyamines and spermines, fatty acid derivatives, peptide nucleic acids (PNAs)

Reagents

Coupling reagents, solvents and scavengers, protecting groups

Resins

Preloaded resins (e.g., based on Trityl, TCP, TentaGel, Methoxybenzhydryl, Merrifield, PAM, Rink, Wang), scavenger resins, hydrazone resins, poly(acrylamide) resins, Cyclover

Linkerology® and Drug Delivery

Linkers for Solid Phase Peptide Synthesis

Cleavable Linkers

Val-Ala-based, Val-Cit-based, disulfide-based, Dde-helping hands, pH-sensitive linkers

Photo-Activatable Linkers

Functionalized Linkers

Clickable linkers, trifunctional linkers, linkers with maleimide function, cross-linkers, selective N-term acylation and biotinylation, 5HP2O

PROTACs

Ligands, linkers & modules

Fullerenes, Poly(2-oxazolines), Dextrans & Plant-Derived Cholesterol

Superparamagnetic Iron Oxide Nanoparticles

Poly-Amino Acids

Poly-Arg, Poly-Glu, Poly-Lys, Poly-Orn, Poly-Sar

PEGylation

Branched PEGylating reagents, (amino-)PEG-acids, PEG-amines & hydrazides & guanidines, reagents for Click-conjugation, Biotin-PEG-reagents, PEG-thiols, PEG-maleimides, other PEGylating reagents

Life Sciences

Biotinylation Reagents

Carbohydrates

Galactose, Glucose, Mannose, Xylose and others

Drug Metabolites

Peptides

Substrates & Inhibitors

E.g., protein kinase inhibitors, substrates for fusion (Halo/Snap/Clip)-tagged proteins

Natural Products

Dyes and Fluorescent Labels

E.g., ICG, AMC, DAPI

Maillard & Amadori Reaction Products

Large portfolio of derivatives useful as standards for food, pharma and cosmetics industry

Vitamins

Custom Synthesis

Your project requires a compound not listed in our portfolio?
Get in contact and inquire about our custom synthesis capabilities.

Our experienced scientists are excited to accept your synthetic challenge!
In such cases, your request undergoes the following stages:

Step-by-Step Analysis

- Customer's demands

Process Evaluation

- Detailed literature review
- Synthetic possibilities

Strategy Development

- Protocol development
- Method development and validation
- Customized synthesis

Quality Consistency

- Identity confirmation
- Purity verification

Our Service Promise

All our services are based on high standards, transparency & documentation, trust, honesty & confidentiality, as well as the required know-how.

High Standards

- Values: sustainability & responsibility
- State-of-the-art equipment & latest technologies
- High quality standards
- Qualified suppliers & regular audits

Transparency & Documentation

- Talk to our specialists – customer care
- Certificates of analysis & origin
- Impurity profiling
- Safety data sheets
- Analytical and process reports

Trust, Honesty & Confidentiality

- Intergenerational business valuing partnerships
- Meeting the customer's expectations
- Integrity towards our customers

Our Know-How

- One-step reactions & complex multi-step synthesis
- Scalability from mg to kg quantities
- Route scouting

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1. Introduction

The naturally low reactivity of functional groups in amino acids makes direct coupling inefficient. To address this, coupling reagents are employed to activate the carboxyl group of one amino acid, enhancing its electrophilic character and promoting nucleophilic attack by the amino group of another.

The primary function of a coupling reagent is to transform the carboxyl group into a more reactive intermediate, typically by incorporating an electron-withdrawing group. This activated species then undergoes a nucleophilic substitution with the free amino group of the incoming amino acid (aminolysis), forming a peptide bond (amide bond) and releasing a by-product. For successful peptide bond formation, the coupling reagent must be highly reactive and selective, minimize undesirable side reactions, and generate soluble by-products that can be easily removed from the reaction mixture.

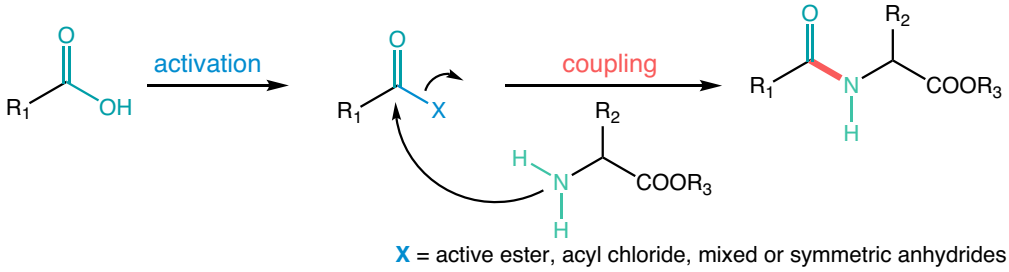

Fig. 1: General mechanism of peptide bond formation.

However, the activation step poses a significant challenge, as there is a risk of racemization, which can proceed *via* two possible pathways (see *Fig. 2*, *Fig. 3*): First, through an intramolecular cyclization reaction that leads to the formation of an oxazolone intermediate (path A, *Fig. 2*). Second, through direct enolization (path B, *Fig. 3*).

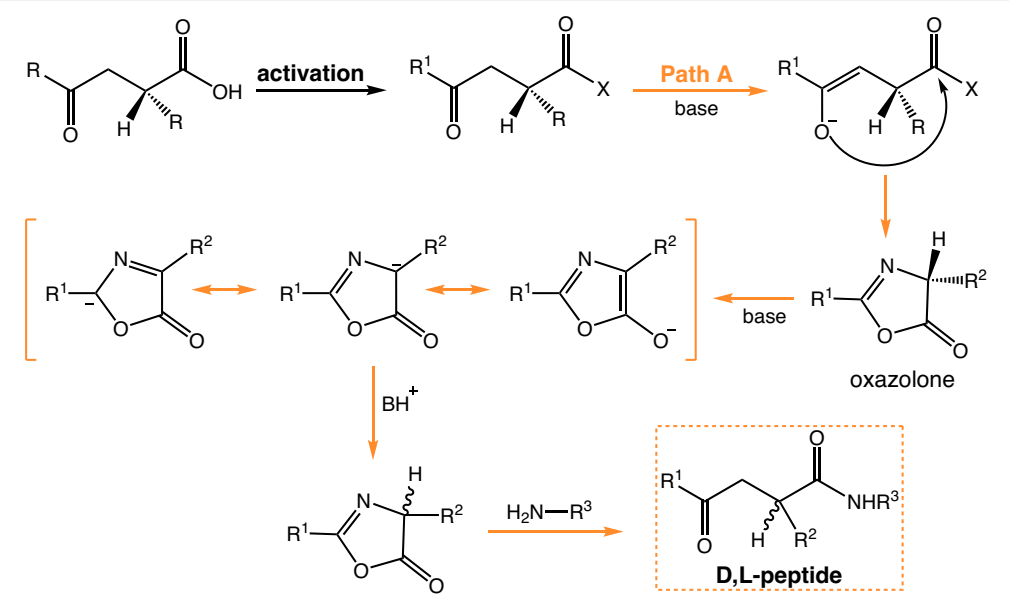

Fig. 2: Pathway A of racemization during amino acid coupling.

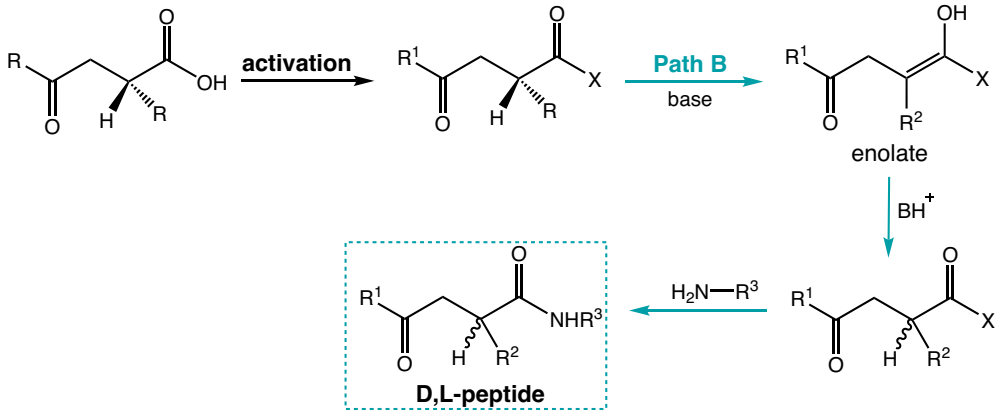

Fig. 3: Pathway B of racemization during amino acid coupling.

This racemization risk is critical in peptide synthesis, as both pathways – cyclization and enolization – can result in the loss of the desired chiral integrity. A careful consideration of reaction conditions is therefore essential. For this purpose, several coupling reagents have been developed, each with unique properties and mechanisms. The most commonly used coupling reagents can be classified into different categories (see *Fig. 4*).

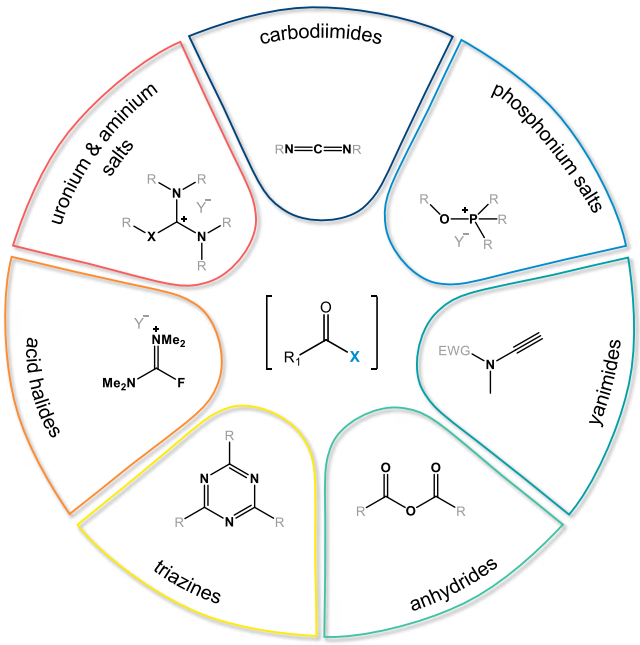

Fig. 4: Overview of selected types of activating reagents.

References:

→ *Peptide Coupling Reagents, More than a Letter Soup; A. El-Faham, F. Albericio;* ***Chem. Rev.*** *2011;* ***111****: 6557-6602. https://doi.org/10.1021/cr100048w*

→ *Recent development in peptide coupling reagents; T. I. Al-Warhi, H. M. A. Al-Hazimi, A. El-Faham;* ***Journal of Saudi Chemical Society*** *2012;* ***16(2)****: 97-116. https://doi.org/10.1016/j.jscs.2010.12.006*

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2. Carbodiimides

In this reaction, a carbodiimide reacts with a carboxylic acid to form *O*-acylurea, a highly reactive intermediate that enables aminolysis (peptide bond formation). In the presence of an excess of the carboxylic acid, the formed *O*-acylurea may also convert into a symmetrical anhydride. These compounds further facilitate peptide bond formation but at a slower rate. *O*-acylisourea has also been demonstrated to undergo cyclization, resulting in the formation of oxazolone, a species that exhibits reduced reactivity and increased propensity for racemization, both of which could potentially compromise the stereochemical integrity of the desired product (see *Fig. 6*).

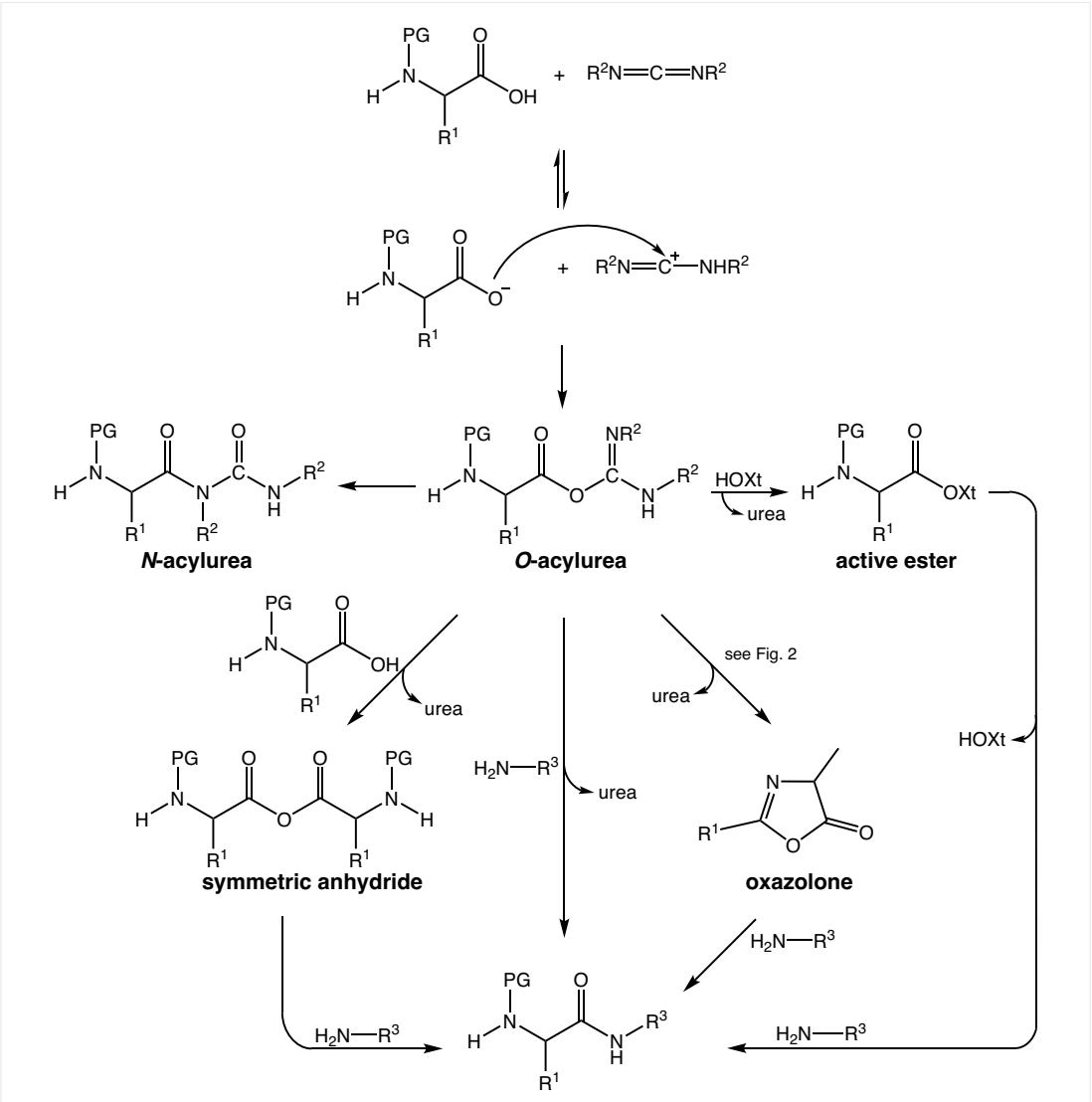

Fig. 5: Carbodiimides as coupling reagents - mechanism and possible side reactions.

The carbodiimide method by applying reagents such as the symmetrical dicyclohexylcarbodiimide (DCC, *RL-1013 on page 9*) and diisopropylcarbodiimide (DIC), *RL-1015 on page 9*) or the unsymmetrical reagents N-ethyl-*N'*-(3-dimethylaminopropyl)carbodiimide (EDC, *RL-1022 on page 9*) or 1-*tert*-butyl-3-ethylcarbodiimide (TBEC, *RL-3910 on page 9*) remains of the most widely used strategies for peptide bond formation.

A significant limitation of using DCC in carbodiimide-mediated couplings is the formation of *N*-acylurea, an unreactive by-product that consumes valuable starting materials without contributing to the peptide yield, e.g., in polar solvents such as DMF. Furthermore, DCC generates dicyclohexylurea (DCU), an insoluble side product that poses challenges for removal, even with chromatographic purification techniques.

These drawbacks have driven the development of alternative carbodiimides, such as diisopropylcarbodiimide (DIC, *RL-1015 on page 9*) which offer an improved solubility in organic solvents.

N-ethyl-*N'*-(3-dimethylaminopropyl)carbodiimide (EDC, *RL-1022 on page 9*) is especially advantageous in solution-phase reactions due to its water-soluble urea by-product which facilitates easier post-reaction workup and simplifies purification, e.g., by extraction methods.

To address challenges with side product formation, racemization and purification, carbodiimides are often used in combination with additives like 1-hydroxybenzotriazole (HOBt) or 1-hydroxy-7-azabenzotriazole (HOAt). These additives form more stable intermediates (active esters) which suppress the formation of *N*-acylurea and reduce racemization (see *Fig. 7*). The enhanced effectiveness of HOBt and HOAt is largely due to their ability to protonate the *O*-acylisourea intermediate, preventing unwanted intramolecular cyclization by forming the mentioned active esters. This shift in the reaction pathway helps to lower the risk of racemization in many cases.

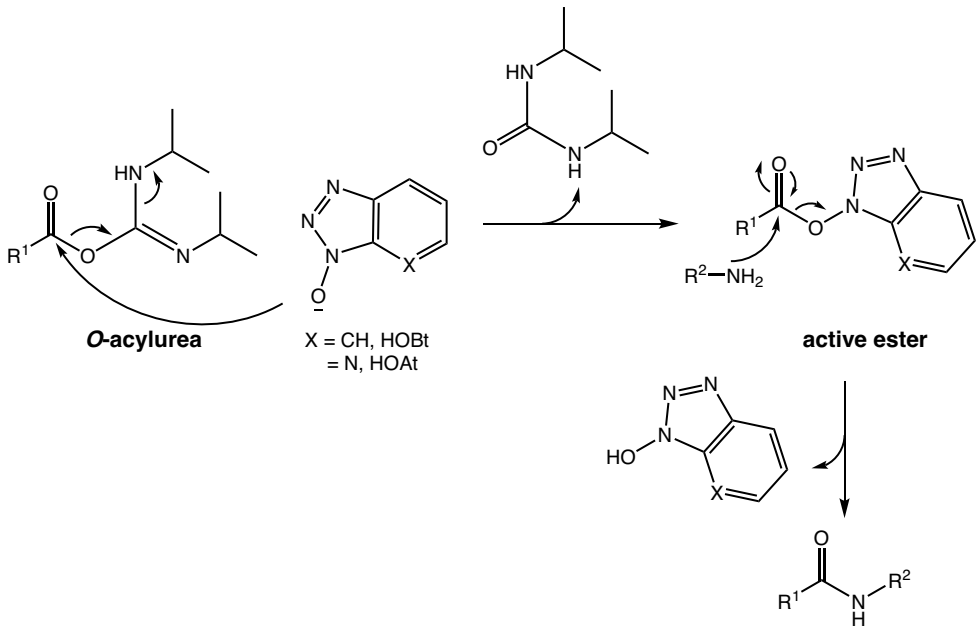

Fig. 7: Mechanism of HOXt coupling.

HOAt offers different advantages over other additives due to the presence of a nitrogen atom at the 7-position of the benzotriazole ring (see *Fig. 8*). This nitrogen atom enhances the ester reactivity through two key mechanisms: first, its electron-withdrawing effect increases the overall reactivity of the intermediate; second, the strategic placement of the nitrogen at position 7 enables a classic neighboring group effect, further accelerating the reaction while preserving the configurational integrity of the peptide product. As a result, HOAt yields higher coupling efficiencies and significantly reduces racemization compared to other additives.

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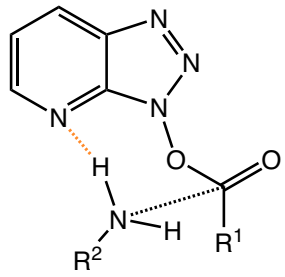

Fig. 8: Neighboring group effect of HOAt.

However, both HOBt and HOAt present safety concerns due to their low molecular weight and the presence of multiple consecutive nitrogen atoms that make them highly explosive. To address this issue, OxymaPure® has been developed as a safer alternative. OxymaPure® (*RL-1180 on page 9*) reduces racemization and side reactions while maintaining a high efficiency in peptide coupling protocols. It forms a stable active ester (see *Fig. 9*) and has been shown to outperform traditional additives like HOBt in many cases, offering a safer and more reliable option for peptide synthesis.

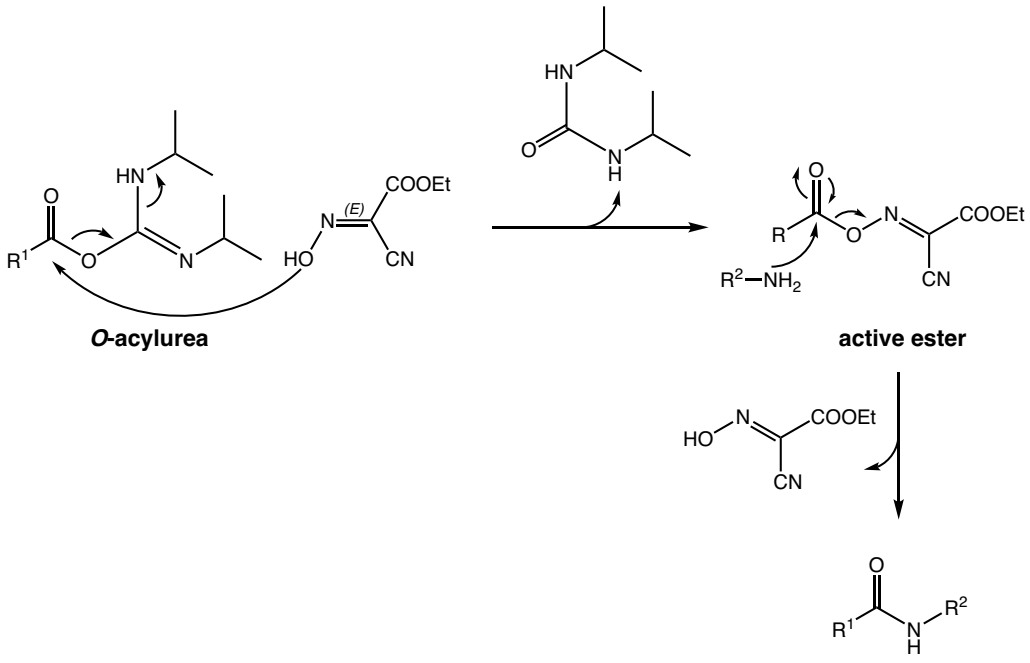

Fig. 9: Coupling mechanism with OxymaPure®.

Despite these benefits, a side reaction has been previously reported where OxymaPure® and DIC form a cyclic adduct, an oxadiazole, resulting in the release of hydrogen cyanide (HCN) as a by-product (see *Fig. 10*).

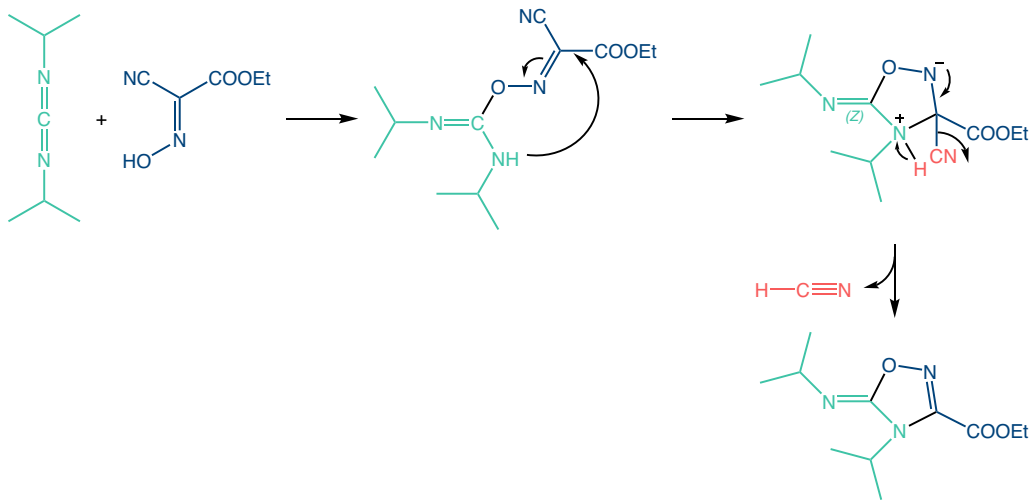

Fig. 10: Side reaction of DIC with OxymaPure®.

This side reaction presents potential safety concerns, but it occurs only to a limited extent. Moreover, new research has demonstrated that the use of asymmetric carbodiimides, such as 1-*tert*-Butyl-3-ethyl-carbodiimide (TBEC, *RL-3910 on page 9*) or EDC (*RL-1022 on page 9*), can effectively suppress the formation of HCN. In this case, instead of a 5-membered oxadiazole ring, a 6-membered oxadiazine ring is formed, reducing the likelihood of HCN release (see *Fig. 11*).

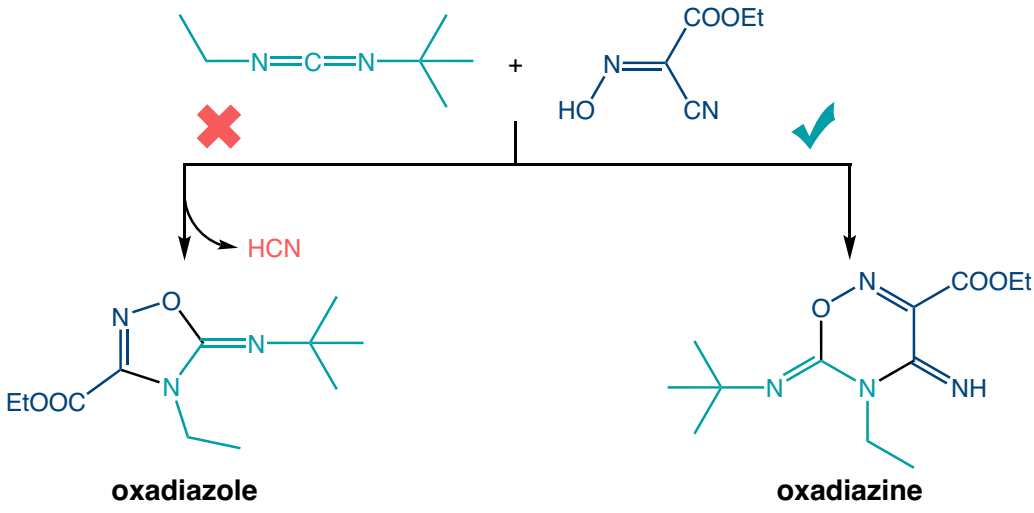

Fig. 11: Limitation of HCN formation by replacement of DIC with TBEC in OxymaPure® coupling mixtures.

Oxyma-B (*RL-2290 on page 9*) is described as an alternative to HOAt, which, compared to OxymaPure®, can reduce racemization, for example in histidine, from 3% to 1%. For large-scale applications of Oxyma-B (*RL-2290 on page 9*), studies on potential side reactions have been conducted. Orlandin and colleagues demonstrated that a carbodiimide-mediated Beckmann rearrangement might occur. This side reaction might lead to a capping of the peptide and therefore limit the yield of the desired peptide. Additionally, this side reaction might cause truncated peptide sequences.

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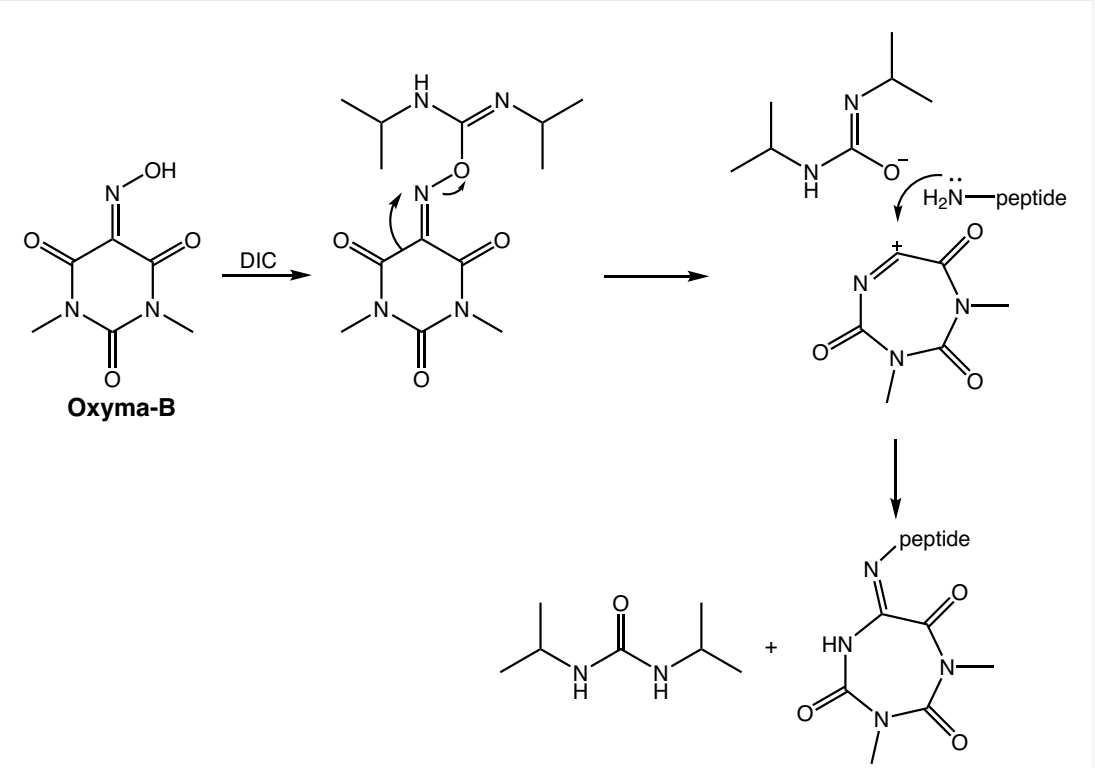

Fig. 12: Proposed mechanism of DIC-mediated Beckmann rearrangement of Oxyma-B.

Guidelines for standard carbodiimide coupling conditions at room temperature

Carbodiimide + additive method

I. In a separate flask or tube, dissolve the protected amino acid (5 eq. relative to the resin loading) and HOXt (5 eq.) in DMF or NMP (5 mL/g of resin).

II. Add carbodiimide (5 eq.) to the solution and stir the mixture for 10 to 20 min for pre-activation.

III. Transfer the activated coupling mixture to the swollen resin and shake the mixture according to the following guidelines:

Conditions	Reaction time [min]
Standard	45
Difficult coupling (e.g., bulky side-chain)	90
After incorporation of the first 20 aa	2 x 45
After sterically-hindered aa	2 x 45

V. Remove the excess reagents after the desired reaction time.

VI. Wash resin with DMF (at least 3 x 5 mL).

Note:

- The active ester formed is unstable and should not be stored; it is recommended to prepare the reaction mixture fresh for each step.
- OxymaPure® (*RL-1180 on page 9*) has been reported to outperform HOBt, though not consistently HOAt. It can also be used with DIC without requiring pre-activation, to avoid HCN formation. As mentioned earlier TBEC (*RL-3910 on page 9*) can serve as a replacement for DIC (*RL-1015 on page 9*). This combination may also eliminate the need for pre-activation in certain cases.
- Coupling protocols for the DIC (*RL-1015 on page 9*) /OxymaPure® (*RL-1180 on page 9*) method using microwave heating are available, and it is advisable to follow the manufacturer's guidelines for optimal results.

References:

→ *Peptide Coupling Reagents, More than a Letter Soup; A. El-Faham, F. Albericio;* ***Chem. Rev.*** *2011;* ***111****: 6557-6602. https://doi.org/10.1021/cr100048w*

→ *Explosive properties of 1-hydroxybenzotriazoles; K. D. Wehrstedt, P. A. Wandrey, D. Heitkamp;* ***J. Hazard. Mater.*** *2005;* ***126****: 1-7, https://doi.org/10.1016/j.jhazmat.2005.05.044*

→ *Understanding OxymaPure as a Peptide Coupling Additive: A Guide to New Oxyma Derivatives; S. R. Manne, A. Sharma, A. Sazonovas, A. El-Faham, B. G. de la Torre, F. Albericio;* ***ACS Omega*** *2022;* ***7(7)****: 6007-6023. https://doi.org/10.1021/acsomega.1c06342*

→ *The carbodiimide method. In The Peptides: Analysis, Synthesis, Biology; D. H. Rich, J. Singh, E. Gross, J. Meienhofer; 1979. Publisher: Academic Press, NY. https://doi.org/10.1016/B978-0-12-304201-9.50011-4*

→ *Peptide Synthesis and Applications in Methods in Molecular Biology; K. J. Jensen, P. T. Shelton, S. L. Pedersen; 2013 Publisher: Humana Totowa, NJ. https://doi.org/10.1007/978-1-62703-544-6*

→ *Eine neue Methode zur Synthese von Peptiden: Aktivierung der Carboxylgruppe mit Dicyclohexylcarbodiimid unter Zusatz von 1-Hydroxy-benzotriazolen; W. König, R. Geiger;* ***Chem Ber.*** *1970;* ***103****: 788-798. https://doi.org/10.1002/cber.19701030319*

→ *1-Hydroxy-7-azabenzotriazole. An efficient peptide coupling additive; L. A. Carpino;* ***J. Am. Chem. Soc.*** *1993;* ***115(10)****: 4397-4398. https://doi.org/10.1021/ja00063a082*

→ *Generation of Hydrogen Cyanide from the Reaction of Oxyma (Ethyl Cyano(hydroxyimino)acetate) and DIC (Diisopropylcarbodiimide), A. D. McFarland, J. Y. Buser, M.C. Embry, C. B. Held, S. P. Kolis;* ***Org. Process Res. Dev.*** *2019;* ***23(9)****: 2099-2105. https://doi.org/10.1021/acs.oprd.9b00344*

→ *Elevating 1-tert-Butyl-3-ethylcarbodiimide (TBEC) as a Reagent for Sustainable Peptide Synthesis: Quality Assessment and Minimizing Racemization, Precipitation, and Radical-Induced Side Reactions by TBEC/Oxyma Couplings in an Environmentally Sensible Solvent; J. Pawlas, J.-H. Choi, C. v. Bargen, S. Maibom-Thomsen, J. H. Rasmussen, O. Ludemann-Hombourger;* ***Org. Process Res. Dev.*** *2023;* ***27(7)****: 1348-1364. https://doi.org/10.1021/acs.oprd.3c00120*

→ *Minimizing HCN in DIC/Oxyma-Mediated Amide Bond-Forming Reactions; M. Erny, M. Lundqvist, J. H. Rasmussen, O. Ludemann-Hombourger, F. Bihel, J. Pawlas;* ***Org. Process Res. Dev.*** *2020;* ***24(7)****: 1341-1349. https://doi.org/10.1021/acs.oprd.0c00227*

→ *tert-Butylethylcarbodiimide as an Efficient Substitute for Diisopropylcarbodiimide in Solid-Phase Peptide Synthesis: Understanding the Side Reaction of Carbodiimides with OxymaPure; S. R. Manne, D. C. Akintayo, O. Luna, A. El-Faham, B. G. de la Torre, F. Albericio;* ***Org. Process Res. Dev.*** *2022;* ***26(10)****: 2894-2899. https://doi.org/10.1021/acs.oprd.2c00220*

→ *Amide Formation: Choosing the Safer Carbodiimide in Combination with Oxyma Pure to Avoid HCN Release; S. R. Manne, O. Luna, G. A. Acosta, M. Royo, A. El-Faham, G. Orosz, B. G. de la Torre, F. Albericio;* ***Org. Lett.*** *2021;* ***23(17)****: 6900-6904. https://doi.org/10.1021/acs.orglett.1c02466*

→ *Oxyma-B, an excellent racemization suppressor for peptide synthesis; Y. E. Jad, S. N. Khattab, B. G. de la Torre, T. Govender, H. G. Kruger, A. El-Faham, F. Albericio;* ***Org. Biomol. Chem.*** *2014;* ***12****: 8379-8385. https://doi.org/10.1039/C4OB01612B*

→ *Carbodiimide-Mediated Beckmann Rearrangement of Oxyma-B as a Side Reaction in Peptide Synthesis; A. Orlandin, I. Guryanov, L. Ferrazzano, B. Biondi, F. Biscaglia, C. Storti, M. Rancan, F. Formaggio, A. Ricci, W. Cabri;* ***Molecules*** *2022;* ***27(13)****: 4235. https://doi.org/10.3390/molecules27134235*

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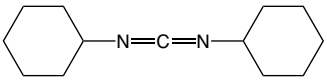
Coupling Reagents

Product details

RL-1013 DCC

N,N'-Dicyclohhexylcarbodiimide

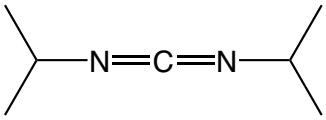
CAS-No. 538-75-0
Formula $C_{13}H_{22}N_2$
Mol. weight 206,3 g/mol



RL-1015 DIC

N,N'-Diisopropylcarbodiimide

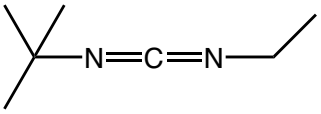
CAS-No. 693-13-0
Formula $C_7H_{14}N_2$
Mol. weight 126,20 g/mol



RL-3910 T-BEC

1-*tert*-Butyl-3-ethylcarbodiimide

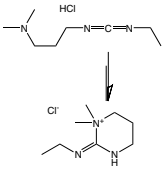
CAS-No. 1433-27-8
Formula $C_7H_{14}N_2$
Mol. weight 126,20 g/mol



RL-1022 EDC*HCl

N-Ethyl-*N'*-(3-dimethylaminopropyl)carbodiimide hydrochloride

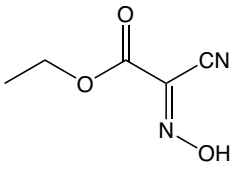
CAS-No. 25952-53-8
Formula $C_8H_{17}N_3$*HCl
Mol. weight 155,24*36,45 g/mol



RL-1180 OxymaPure

Ethyl cyano(hydroxyimino)acetate, Ethyl cyano-glyoxylate-2-oxime

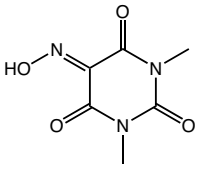
CAS-No. 3849-21-6
Formula $C_5H_6N_2O_3$
Mol. weight 142,11 g/mol



RL-2290 Oxyma-B

5-(Hydroxyimino)1,3-dimethylpyrimidi-ne-2,4,6-(1H,3H,5H)-trione

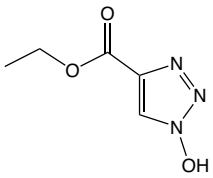
CAS-No. 5417-13-0
Formula $C_6H_7N_3O_4$
Mol. weight 185,14 g/mol



RL-1091 HOCt

Ethyl 1-hydroxy-1H-1,2,3-Triazole-4-carboxylate

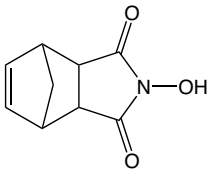
CAS-No.	137156-41-3
Formula	$C_5H_7N_3O_3$
Mol. weight	157,13 g/mol



RL-1036 HONB

N-Hydroxy-5-norbornene-2,3-dicarboxylimide

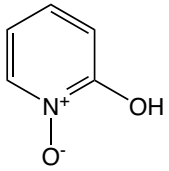
CAS-No.	21715-90-2
Formula	$C_9H_9NO_3$
Mol. weight	179,17 g/mol



RL-2002 HOPO

2-Hydroxypyridine-N-oxide

CAS-No.	13161-30-3
Formula	$C_5H_5NO_2$
Mol. weight	111,10 g/mol



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3. Phosphonium Salts

Phosphonium salts activate the carboxyl group of the amino acid by forming a phosphonium or benzotriazolyl ester as the activated intermediate. This compound in turn reacts with the amine group of the growing peptide chain to form an amide bond, with minimal side reactions. These couplings are performed in the presence of at least 1 equivalent (eq.) of base (most commonly 2 eq. of diisopropylethylamine (DIPEA) or *N*-methylmorpholine (NMM) are used), as the phosphonium-based reagents react with a carboxylate. It is noteworthy that unlike carbodiimide-based couplings, phosphonium salts avoid urea formation thereby reducing the likelihood of side product generation.

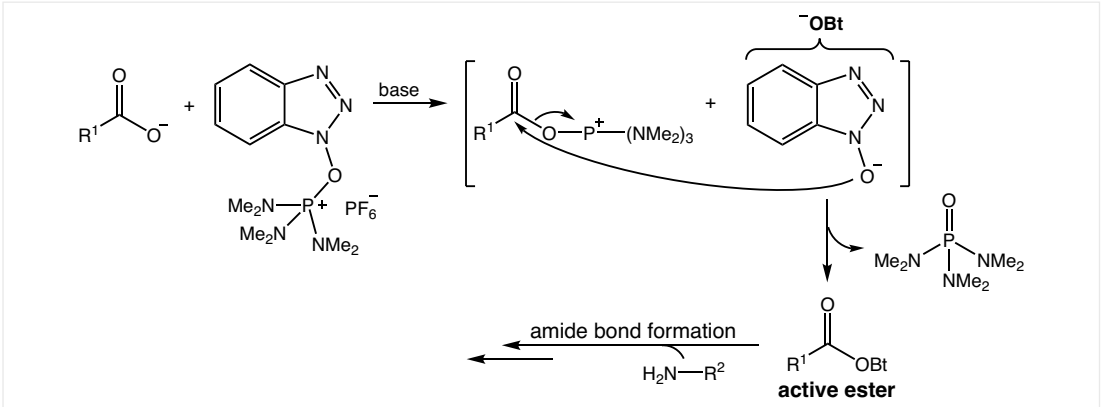

Fig. 13: BOP-mediated coupling mechanism.

Benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP, *RL-5096 on page 14*) was the first phosphonium salt introduced for peptide synthesis. However, its use raised safety concerns due to the formation of hexamethylphosphoramide (HMPA), a carcinogenic side product (see *Fig. 13*). As a result, safer alternatives like benzotriazol-1-yloxytri(pyrrolidino) phosphonium hexafluorophosphate (PyBOP, *RL-2005 on page 15*) and [(7-azabenzotriazol-1-yl)oxy]tris(pyrrolidino) phosphonium hexafluorophosphate (PyAOP, *RL-1210 on page 15*) were developed in which the dimethylamine moiety was replaced by pyrrolidine to avoid HMPA formation. These newer reagents gained popularity due to their lower risk of racemization in comparison to the carbodiimide method and high efficiency in challenging peptide couplings. Bromotri(pyrrolidino) phosphonium hexafluorophosphate (PyBrOP, *RL-1051 on page 15*) and (6-chloro-benzotriazol-1-yloxy)tris(pyrrolidino) phosphonium hexafluorophosphate (PYCLOCK, *RL-2180 on page 15*) are described in the coupling of *N*-methylated amino acids.

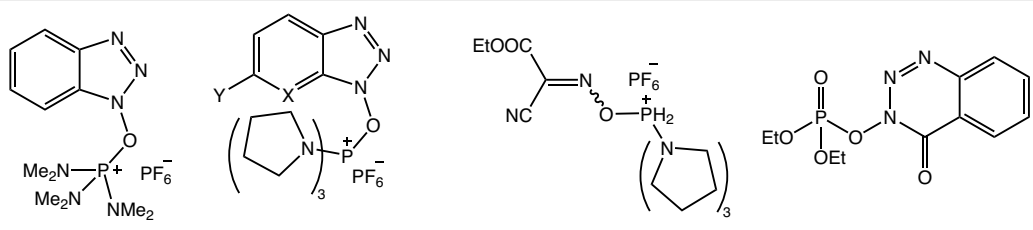

Fig. 14: Selection of phosphonium-based coupling reagents.

After benzotriazoles like HOBt and HOAt were classified as explosive, and OxymaPure® (*RL-1180 on page 9*) was introduced as a safer alternative, *O*-[(cyano(ethoxycarbonyl)methyliden)-amino]-yloxytri(pyrrolidino) phosphonium hexafluorophosphate (PyOxim, *RL-2270 on page 14*), an OxymaPure®-based phosphonium salt was developed. This new reagent was designed to offer improved safety while maintaining high efficiency in peptide coupling reactions.

3-(Diethoxyphosphoryloxy)-1,2,3-benzotriazin-4(3H)-one (DEPBT, *RL-1153 on page 14*) is an organophosphorus compound that is described as a coupling reagent in the synthesis of bioactive peptides with a great resistance to racemization.

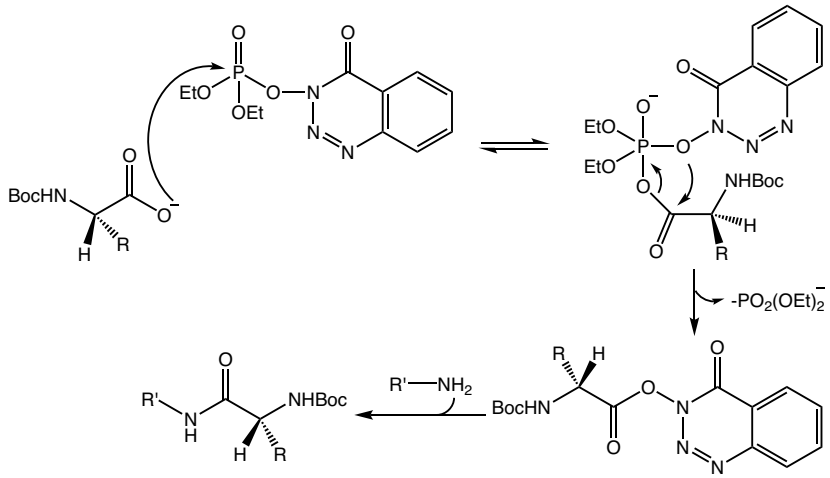

Fig. 15: Proposed mechanism for DEPBT-mediated coupling.

Guidelines for standard phosphonium-based coupling conditions

BOP (*RL-5096 on page 14*)

I. In a separate flask or tube, dissolve the *N*-protected amino acid (2 eq. relative to the resin loading) in DMF (5 mL/g of resin) and add it to the pre-swelled resin.

II. Add BOP (2 eq., 1 M solution in DMF) and DIPEA or NMM (4 eq.) to the solution. Note: In order to suppress racemization HOBt or OxymaPure® (2 eq., 0.5 M solution in DMF) can be added.

III. Shake the resin with the coupling mixture for 10-60 min.

IV. Remove the excess reagents after the desired reaction time.

V. Wash the resin with DMF (at least 3 x 5 mL).

PyBOP (*RL-2005 on page 15*)

I. In a separate flask or tube, dissolve the *N*-protected amino acid (1.1 eq. relative to the resin loading) in DMF (5 mL/g of resin) and add it to the pre-swelled resin.

II. Add PyBOP (1.1 eq., 1 M solution in DMF) and DIPEA or NMM (2 eq.) to the solution. Note: In order to suppress racemization HOBt or OxymaPure® (1.1 eq., 0.5 M solution in DMF) can be added.

III. Shake the resin with the coupling mixture for 30-60 min.

IV. Remove the excess reagents after the desired reaction time.

V. Wash the resin with DMF (at least 3 x 5 mL).

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PyBrOP (*RL-1051 on page 15*) or PyCLOCK (*RL-2180 on page 15*) for *N*-methylated amino acids

I. In a separate flask or tube, dissolve the *N*-protected amino acid (2 eq. relative to the resin loading) in DMF or DCM (5 mL/g of resin) and add it to the resin.
II. Add PyBrOP or PyCLOCK (1.1 eq., 1 M solution in DMF) and cool the solution to 0°C.
III. Add DIPEA (6 eq.) to the reaction mixture.
IV. Shake the resulting reaction mixture for 1 min at 0°C followed by 60 min at room temperature.
V. Remove the excess reagents after the desired reaction time.
VI. Wash the resin with DMF (at least 3 x 5 mL).

PyOxim (*RL-2270 on page 14*)

I. In a separate flask or tube, dissolve the *N*-protected amino acid (3 eq. relative to the resin loading) in DMF or NMP (5 mL/g of resin).
II. Add PyOxim (3 eq.) and DIPEA (6 eq.) to the solution.
III. Shake the resulting reaction mixture for 1-2 min (pre-activation).
IV. Add the solution to the resin and allow the coupling to proceed for 30 min.
V. Remove the excess reagents after the desired reaction time.
VI. Wash the resin with DMF (at least 3 x 5 mL).

DEPBT (*RL-1153 on page 14*) (solid phase)

I. In a separate flask or tube, dissolve the *N*-protected amino acid (1.5 eq. relative to the resin loading) in DCM or DMF (5 mL/g resin) and add it to the pre-swelled resin.
II. Add DIPEA or NEt_3 (3 eq.).
III. Add DEPBT (1.5 eq.).
IV. Shake the resulting reaction mixture for 1 to 2 h.
V. Remove the excess reagents after the desired reaction time.
VI. Wash the resin with DMF or DCM (at least 3 x 5 mL).

DEPBT (*RL-1153 on page 14*) (solution phase)

I. Dissolve the protected amino acid and amino acid ester in THF or DMF (2 mL/mmol).
II. Add DEPBT (1.1 eq.).
III. Add NEt_3 (2 eq.).
IV. Shake the resulting reaction mixture for 2 to 6 h.
V. Pour the mixture into a saturated sodium chloride solution (40 mL/mmol) and extract the precipitate with ethyl acetate (3 x 50 mL).
VI. In case of THF: triethylamine hydrochloride is removed by filtration.
VII. Remove the solvent *in vacuo*.

References:

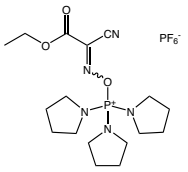
- *Acyloxyphosphonium salts as acylating agents. Synthesis of peptides; G. Gawne, G.W. Kenner, R.C. Sheppard;* ***J. Am. Chem. Soc.*** *1969;* ***91(20)****: 5669-5670.* https://doi.org/10.1021/ja01048a057
- *PyBOP®: A new peptide coupling reagent devoid of toxic by-product; J. Coste, D. Le-Nguyen, B. Castro;* ***Tetrahedron Letters*** *1990;* ***31(2)****: 205-208.* https://doi.org/10.1016/S0040-4039(00)94371-5
- *Oxybenzotriazole free peptide coupling reagents for N-methylated amino acids; J. Coste, E. Frérot, P. Jouin, B.Castro;* ***Tetrahedron Letters*** *1991;* ***31(17)****: 1967-1970.* https://doi.org/10.1016/0040-4039(91)85015-W
- *3-(Diethoxyphosphoryloxy)-1,2,3- benzotriazin-4(3H)-one (DEPBT): A New Coupling Reagent with Remarkable Resistance to Racemization; H. Li, X. Jiang, Y. Ye, C. Fan, T. Romoff, M. Goodman;* ***Org. Lett.*** *1999;* ***1(1)****: 91-94.* https://doi.org/10.1021/ol990573k
- *New trends in peptide coupling reagents; F. Albericio, R. Chinchilla, D.J. Dodsworth, C. Nájera;* ***Organic Preparations and Procedures International*** *2009;* ***33(3)****: 203-303.* https://doi.org/10.1080/00304940109356592
- *BOP-OXy, BOP-OBt, and BOP-OAt: novel organophosphinic coupling reagents useful for solution and solid-phase peptide synthesis; A. El-Faham, S. N. Khattab, R. Subirós-Funosas, F. Albericio;* ***J Pept Sci*** *2014;* ***20(1)****: 1-6.* https://doi.org/10.1002/psc.2579
- *PyOxP and PyOxB: the Oxyma-based novel family of phosphonium salts; R. Subirós-Funosas, A. El-Faham, F. Albericio;* ***Org. Biomol. Chem.*** *2010;* ***8****: 3665-3673.* https://doi.org/10.1039/C003719B

Product details

RL-2270 PyOxim

[Ethyl cyano(hydroximino)acetato-O]tri-1-pyrrolidinyl-phosphonium hexafluorophosphate

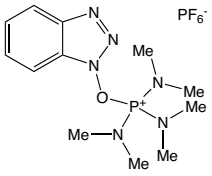
CAS-No.	153433-21-7
Formula	$C_{17}H_{29}N_5O_3P^*PF_6$
Mol. weight	382,42*144,96 g/mol



RL-5096 BOP

Benzotriazol-1-yl-oxy-tris-(dimethylamino)-phosphonium hexafluorphosphate

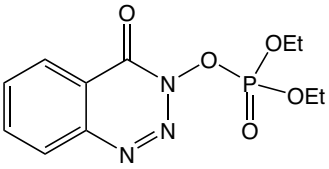
CAS-No.	56602-33-6
Formula	$C_{12}H_{22}N_6OP^*PF_6$
Mol. weight	297,32*144,96 g/mol



RL-1153 DEPBT

3-(Diethoxyphosphoryloxy)-1,2,3-benzotriazin-4-(3H)-one

CAS-No.	165534-43-0
Formula	$C_{11}H_{14}N_3O_5P$
Mol. weight	299,23 g/mol



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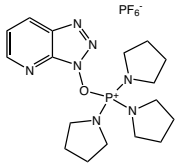
Coupling Reagents

Product details

RL-1210 PyAOP

(3-Hydroxy-3H-1,2,3-triazolo[4,5-b]pyridinato-O) tri-1-pyrrolidinyl-phosphorus hexafluorophosphate

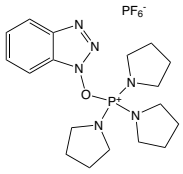
CAS-No.	156311-83-0
Formula	$C_{17}H_{27}N_7OP^*PF_6$
Mol. weight	376,42*144,96 g/mol



RL-2005 PyBOP

Benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphat

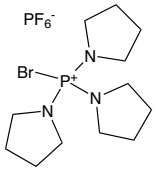
CAS-No.	128625-52-5
Formula	$C_{18}H_{28}N_6OP^*PF_6$
Mol. weight	375,43*144,96 g/mol



RL-1051 PyBrOP

Bromo-tris-pyrrolidino-phosphonium hexafluoro-phosphat

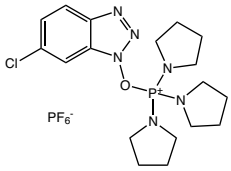
CAS-No.	132705-51-2
Formula	$C_{12}H_{24}N_3Br^*PF_6$
Mol. weight	321,22*144,96 g/mol



RL-2180 PyCLOCK

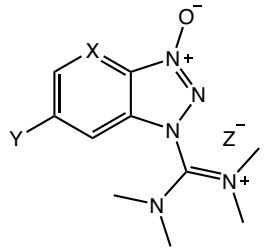
(6-Chlorobenzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate

CAS-No.	893413-42-8
Formula	$C_{18}H_{27}ClN_6OP^*PF_6$
Mol. weight	409,87*144,96 g/mol

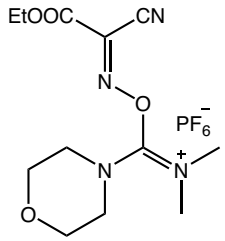


4. Uronium and Aminium Salts

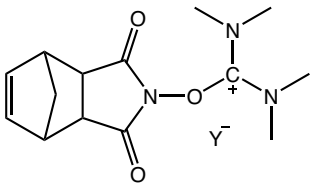
Uronium and aminium salts, commonly represented by *O*-(benzotriazol-1-yl)-1,1,3,3-tetramethyluroronium hexafluorophosphate (HBTU, *RL-1030 on page 20*), *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU, *RL-1190 on page 20*), O-benzotriazol-1-yl-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU, *RL-1060 on page 21*), and 1-[(1-(cyano-2-ethoxy-2-oxoethylideneamino-oxy)-dimethylamino-morpholinomethylene)]methanaminium hexafluorophosphate (COMU, *RL-1175 on page 20*), are derived from carbodiimides (see *Fig. 16*). Instead of a phosphonium residue they bear a positive charged carbon or nitrogen atom. Typically, tetrafluoroborate (BF_4^-) or hexafluorophosphate (PF_6^-) anions are used as non-nucleophilic counter ions. In comparative studies it was revealed that the counterion did not exert a significant influence on the rate of coupling or the rate of racemization. The first described compounds were HOBt derivatives analogous to the phosphonium-based coupling reagents.



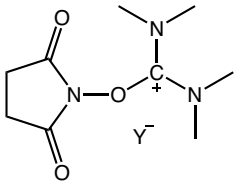
HATU (X = N, Y = H, Z = PF_6^-)
HBTU (X =CH, Y = H, Z = PF_6^-)
HCTU (X = CH, Y = Cl, Z = PF_6^-)
TBTU (X =CH, Y = H, Z = BF_4^-)



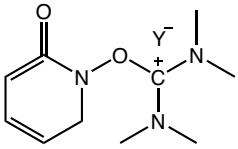
COMU



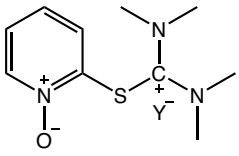
HNTU (Y = PF_6^-)
TNTU (Y = BF_4^-)



HSTU (Y = PF_6^-)
TSTU (Y = BF_4^-)



HPTU (Y = PF_6^-)
TPTU (Y = BF_4^-)



HOTT (Y = PF_6^-)
TOTT (Y = BF_4^-)

Fig. 16: Selection of uronium and aminium salt-based coupling reagents; HATU = O-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; HBTU = O-(benzotriazol-1-yl)-1,1,3,3-tetramethyluroronium hexafluorophosphate; HCTU = O-(6-chlorobenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate; TBTU = O-benzotriazol-1-yl-1,1,3,3-tetramethyluronium tetrafluoroborate; COMU = 1-[(1-(cyano-2-ethoxy-2-oxoethylideneamino-oxy)-dimethylamino-morpholinomethylene)]methanaminium hexafluorophosphate; HNTU = 2-(5-norbornene-2,3-dicarboximido)-1,1,3,3-tetramethyluronium hexafluorophosphate; TNTU = 2-(5-norbornene-2,3-dicarboximido)-1,1,3,3-tetramethyluronium tetrafluoroborate; HSTU = 2-succinimido-1,1,3,3-tetramethyluronium hexafluorophosphate; TSTU = 2-succinimido-1,1,3,3-tetramethyluronium tetrafluoroborate; HPTU = 2-(2-oxo-1(2H)-pyridyl-1,1,3,3-tetramethyluronium hexafluorophosphate; TPTU = 2-(2-oxo-1(2H)-pyridyl-1,1,3,3-tetramethyluronium tetrafluoroborate; HOTT = S-(1-oxido-2-pyridinyl)-1,1,3,3-tetramethylthiouronium hexafluorophosphate; TOTT = S-(1-oxido-2-pyridinyl)-1,1,3,3-tetramethylthiouronium tetrafluoroborate.

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Like phosphonium salts uronium/aminium salts work in combination with a base such as DIPEA to activate the carboxyl function of the incoming amino acid (see *Fig. 17*).

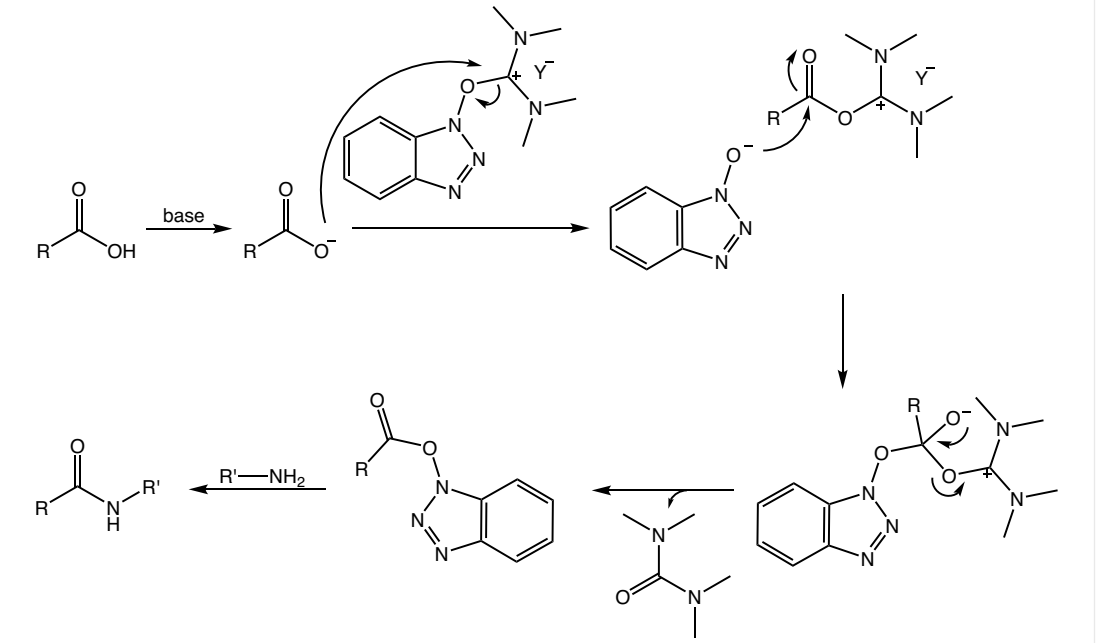

Fig. 17: Coupling mechanism of uronium reagents: The example of HBTU (RL-1030 on page 20).

However, these reagents might also directly react with the free amino function to form a guanidine by-product, which in turn leads to truncated peptide sequences and reduces the yield of the desired peptide (see *Fig. 18*).

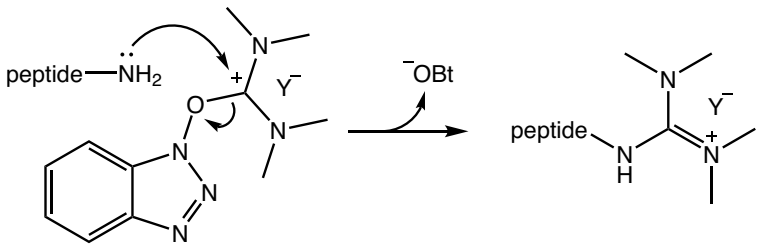

Fig. 18: Guanidinium formation with uronium/aminium-based reagents.

Another consideration is the stability of these reagents upon storage. Uronium/aminium salts are sensitive to moisture and require careful handling as well as storage to maintain their reactivity. In some cases, degradation can lead to lower yields or the need for higher concentrations of reagents.

The HOSu (*N*-hydroxysuccinimide)-based coupling reagents 2-(5-norbornene-2,3-dicarboximido)-1,1,3,3-tetramethyluronium tetrafluoroborate, (TNTU, *RL-1063 on page 21*) and 2-succinimido-1,1,3,3-tetramethyluronium tetrafluoroborate, (TSTU, *RL-1067 on page 22*) have been shown to be useful coupling reagents under aqueous reaction conditions.

S-(1-oxido-2-pyridinyl)-1,1,3,3-tetramethylthiouronium hexafluorophosphate, HOTT, *RL-1156 on page 21*) and S-(1-oxido-2-pyridinyl)-1,1,3,3-tetramethylthiouronium tetrafluoroborate, (TOTT, *RL-1159 on page 22*), derived from 1,1,3,3-tetramethylurea, are used in solution-phase peptide synthesis. These compounds demonstrate comparable results to more expensive coupling reagents in terms of both yield and racemization rates.

2-(2-Oxo-1(2H)-pyridyl-1,1,3,3-tetramethyluronium tetrafluoroborate (TPTU, *RL-1064 on page 22*) is classified as a potentially explosive but not shock-sensitive compound. However, it is also applied in large-scale syntheses, such as the synthesis of an aza-dipeptide analog used in the treatment of AIDS.

Guidelines for uronium/aminium-based coupling procedures

HBTU (*RL-1030 on page 20*) / TBTU (*RL-1060 on page 21*) / HCTU (*RL-1031 on page 20*) / HATU (*RL-1190 on page 20*)

I. In a separate flask or tube, dissolve the *N*-protected amino acid (2 eq. relative to the resin loading) in DMF (5 mL/g of resin) and add it to the pre-swelled resin.
II. Add coupling reagent (2 eq.) and DIPEA (4 eq.) to the solution.
Note: In case of HATU (*RL-1190 on page 20*) use 1.9 eq. of coupling reagent but 4 eq. of base.
III. In order to suppress racemization HOBt or OxymaPure® (*RL-1180 on page 9*) (2 eq., 0.5 M solution in DMF) can be added.
IV. Shake the resin with the coupling mixture for 10-60 min.
V. Remove the excess reagents after the desired reaction time.
VI. Wash the resin with DMF (at least 3 x 5 mL).

COMU (*RL-1175 on page 20*) (solid phase)

I. In a separate flask or tube, dissolve the *N*-protected amino acid (3 eq. relative to the resin loading) in DMF (5 mL/g of resin) with COMU (*RL-1175 on page 20*) (3 eq., 0.3 M in DMF) and DIPEA (6 eq.).
II. Pre-activate the mixture for 1 min and add it to the pre-swelled resin.
III. Shake the resin with the coupling mixture for 10-30 min.
IV. Remove the excess reagents after the desired reaction time.
V. Wash the resin with DMF (at least 3 x 5 mL).
VI. Repeat the procedure in case of sterically-hindered amino acids.

COMU (*RL-1175 on page 20*) (solution phase)

I. Add (0.25 mmol) COMU (*RL-1175 on page 20*) to a mixture of *N*-protected amino acid (0.25 mmol), the amino component (0.25 mmol) and base (0.50 mmol or 0.75 mmol in case of ester hydrochloride) in 2 mL DMF at 0 °C.
II. Stir the reaction mixture for 1 h at 0°C and 2-3 h at room temperature.
III. After complete reaction, dilute the reaction mixture with EtOAc (25 mL).
IV. Extract with 1N HCl (2 x 5 mL), 1N $NaHCO_3$ (2 x 5 mL) and sat. NaCl solution (2 x 5 mL).
V. Dry the organic phase with $MgSO_4$ and remove the solvent *in vacuo*.

TSTU (*RL-1067 on page 22*) / HSTU (*RL-1039 on page 21*) / TNTU (*RL-1063 on page 21*) / HNTU (*RL-1155 on page 21*) (solution phase)

I. Dissolve *N*-protected amino acid (1.0 mmol) and of coupling reagent (1.5 mmol) with DIPEA (2 mmol) and amino acid component (2 mmol) in 4 mL DMF.
II. Stir the reaction for 10 min at room temperature (TLC control).
III. After complete reaction, dilute the reaction mixture with H_2O (25 mL).
IV. Extract with DCM (3 x 25 mL).
V. Dry the organic phase with Na_2SO_4 and remove the solvent *in vacuo*.

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HOTT (*RL-1156 on page 21*) / TOTT (*RL-1159 on page 22*) (solution phase)

I. Dissolve *N*-protected amino acid (1 mmol) and the amino component (1 mmol) in 5 mL DMF.
II. Add DIPEA (2 mmol) and coupling reagent (1 mmol) to the mixture.
III. Stir the reaction for 30 min at room temperature (TLC control).
IV. After complete reaction, dilute the reaction mixture with sat. NaCl (25 mL).
V. Extract with EtOAc (3 x 25 mL).
VI. Wash the combined organic phases with 2N HCl (2 x 10 mL), sat. $NaHCO_3$ (2 x 10 mL) and H_2O (6 x 10 mL).
VII. Dry the organic phase with Na_2SO_4 and remove the solvent *in vacuo*.

TPTU (*RL-1064 on page 22*) (solution phase)

I. Dissolve *N*-protected amino acid (3 eq.) and TPTU (*RL-1064 on page 22*) (3 eq.) in DCM (3 mL, overall concentration 1 M).
II. Cool the resulting solution to 0°C.
III. Add DIPEA (6 eq.) to the mixture.
IV. Add the amino component (3 eq.) to the generated active ester portion wise at 0-5 °C.
V. Remove the ice bath and let the reaction mixture stir at room temperature overnight.
VI. Wash the reaction mixture with H_2O (10mL), sat. $NaHCO_3$ (10 mL), and sat. NaCl (5 mL).
VII. Extract the aqueous phases with DCM (2 x 5 mL).
VIII. Dry the organic phase with Na_2SO_4 and remove the solvent *in vacuo*.

References:

→ *Use of Onium Salt-Based Coupling Reagents in Peptide Synthesis; F. Albericio, J. M. Bofill, A. El-Faham, S. A. Kates;* ***J. Org. Chem.*** *1998, 63: 9678-9683. https://doi.org/10.1021/jo980807y*
→ *Understanding the structure/reactivity of aminium/uronium salts as coupling reagents in peptide synthesis; J. M. Bofill, F. Albericio;* ***Tetrahedron Letters*** *1999;* ***40(13)****: 2641-2644. https://doi.org/10.1016/S0040-4039(99)00262-2*
→ *The Uronium/Guanidinium Peptide Coupling Reagents: Finally the True Uronium Salts; L. A. Carpino, H. Imazumi, A. El-Faham, F. J. Ferrer, C. Zhang, Y. Lee, B. M. Foxman, P. Henklein, C. Hanay, C. Mügge, H. Wenschuh, J. Klose, M. Beyermann, M. Bienert;* ***Angew. Chem. Int. Ed.*** *2002;* ***41(3)****: 441-445. https://doi.org/10.1002/1521-3773(20020201)41:3<441::AID-ANIE441>3.0.CO;2-N*
→ *Morpholine-Based Immonium and Halogenoamidinium Salts as Coupling Reagents in Peptide Synthesis; A. El-Faham, F. Albericio;* ***J. Org. Chem.*** *2008;* ***73(7)****: 2731-2737. https://doi.org/10.1021/jo702622c*
→ *Amide bond formation: beyond the myth of coupling reagents; E. Valeur, M. Bradley;* ***Chem. Soc. Rev.*** *2009;* ***38****: 606-631. https://doi.org/10.1039/b701677h*
→ *COMU: A Safer and More Effective Replacement for Benzotriazole-Based Uronium Coupling Reagents; A. El-Faham, R. S. Funosas, R. Prohens, F. Albericio;* ***Chemistry – A European Journal*** *2009;* ***15(37)****: 9404-9416. https://doi.org/10.1002/chem.200900615*
→ *COMU: A third generation of uronium-type coupling reagents; A. El-Faham, F. Albericio;* ***J. Pept. Sci.*** *2010;* ***16****: 6-9. https://doi.org/10.1002/psc.1204*
→ *Re-evaluating the stability of COMU in different solvents; A. Kumar, Y. E. Jad, B. G. de la Torre, A. El-Faham, F. Albericio;* ***J. Pept. Sci.*** *2017;* ***23(10)****: 763-768. https://doi.org/10.1002/psc.3024*
→ *Fast conventional Fmoc solid-phase peptide synthesis: a comparative study of different activators; C. A. Chantell, M. A. Onaiyekan, M. Menakuru;* ***J. Pept. Sci.*** *2012;* ***18(2)****: 88-91. https://doi.org/10.1002/psc.1419*
→ *A comparative study of amide-bond forming reagents in aqueous media – Substrate scope and reagent compatibility; M. Badland,, R. Crook, B. Delayre, S. J. Fussell, I. Gladwell, M. Hawksworth, R. M. Howard, R. Walton, G. A. Weisenburger;* ***Tetrahedron Letters*** *2017;* ***58(46)****: 4391-4394. https://doi.org/10.1016/j.tetlet.2017.10.014*
→ *New coupling reagents in peptide chemistry; R. Knorr, A. Trzeciak, W. Bannwarth, D. Gillessen;* ***Tetrahedron Letters*** *1989;* ***30(15)****: 1927-1930. https://doi.org/10.1016/S0040-4039(00)99616-3*
→ *2-Mercaptopyridine-1-oxide-based peptide coupling reagents; F. Albericio, M. A. Bailén, R. Chinchilla, D. J. Dodsworth, C. Nájera;* ***Tetrahedron*** *2001;* ***57(47)****: 9607-9613. https://doi.org/10.1016/S0040-4020(01)00985-1*

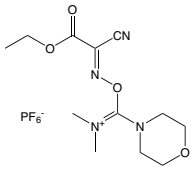
→ *New Aza-Dipeptide Analogues as Potent and Orally Absorbed HIV-1 Protease Inhibitors: Candidates for Clinical Development; G. Bold, A. Fässler, H.-G.Capraro, R. Cozens, T. Klimkait, J. Lazdins, J. Mestan, B. Poncioni, J. Rösel, D. Stover, M. Tintelnot-Blomley, F. Acemoglu, W. Beck, E. Boss, M. Eschbach, T. Hürlimann, E. Masso, S. Roussel, K. Ucci-Stoll, D. Wyss, M. Lang;* ***J. Med. Chem.*** *1998;* ***41(18)****: 3387-3401.* https://doi.org/10.1021/jm970873c

Product details

RL-1175 COMU ®

1-[1-(Cyano-2-ethoxy-2-oxoethylideneaminooxy)-dimethylamino-morpholino]-uronium hexafluorophosphate

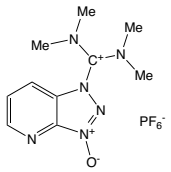
CAS-No.	1075198-30-9
Formula	$C_{12}H_{19}N_4O_4PF_6$
Mol. weight	428,26 g/mol



RL-1190 HATU

2-(1H-7-Azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronoium hexafluorphosphate

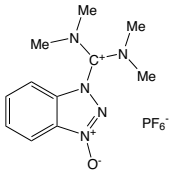
CAS-No.	148893-10-1
Formula	$C_{10}H_{15}N_6O^*PF_6$
Mol. weight	235,27*144,96 g/mol



RL-1030 HBTU

2-(1H-Benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate

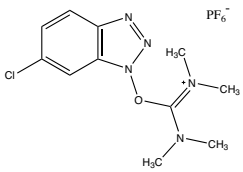
CAS-No.	94790-37-1
Formula	$C_{11}H_{16}N_5O^*PF_6$
Mol. weight	234,28*144,96 g/mol



RL-1031 HCTU

O-(1H-6-Chlorobenzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate

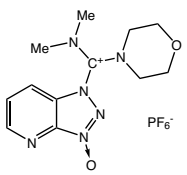
CAS-No.	330645-87-9
Formula	$C_{11}H_{15}ClF_6N_5OP$
Mol. weight	413,69 g/mol



RL-2170 HDMA

1-[(Dimethylamino)(morpholino)methylene]-1H-[1,2,3]triazolo[4,5-b]pyridine-1-ium 3-oxide hexafluorophosphate

CAS-No.	958029-37-3
Formula	$C_{12}H_{17}N_6O_2{}^*PF_6$
Mol. weight	277,30*144,96 g/mol



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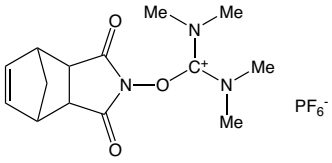
Coupling Reagents

Product details

RL-1155 HNTU

2-(endo-5-norbornene-2,3-dicarboxymido)-1,1,3,3-tetramethyluronium hexafluorophosphate

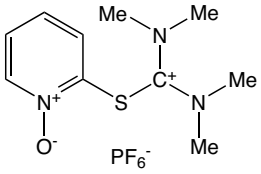
CAS-No. 208462-94-6
Formula $C_{14}H_{20}N_3O_3{*}PF_6$
Mol. weight 278,33*144,96 g/mol



RL-1156 HOTT

S-(1-Oxido-2-pyridyl)-thio-N,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate

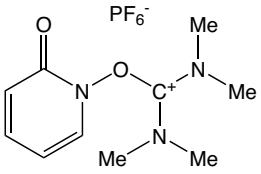
CAS-No. 212333-72-7
Formula $C_{10}H_{16}N_3OS{*}PF_6$
Mol. weight 226,32*144,96 g/mol



RL-1092 HPTU

O-(1,2-Dihydro-2-oxo-pyridyl]-N,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate

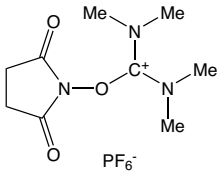
CAS-No. 364047-51-8
Formula $C_{10}H_{16}N_3O_2{*}PF_6$
Mol. weight 210,26*144,96 g/mol



RL-1039 HSTU

2-Succinimido-1,1,3,3-tetramethyluronium hexafluorophosphate

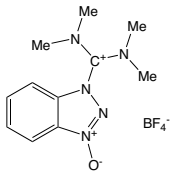
CAS-No. 265651-18-1
Formula $C_9H_{16}N_3O_3{*}PF_6$
Mol. weight 359,21 g/mol



RL-1060 TBTU

2-(1H-Benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate

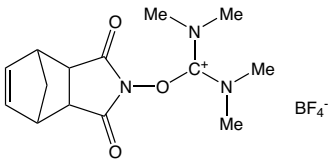
CAS-No. 125700-67-6
Formula $C_{11}H_{16}N_5O{*}BF_4$
Mol. weight 234,28*86,81 g/mol



RL-1063 TNTU

2-(endo-5-norbornene-2,3-dicarboxymido)-1,1,3,3-tetramethyluronium tetrafluoroborate

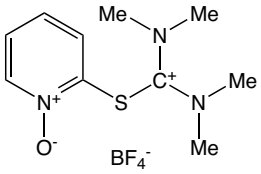
CAS-No. 125700-73-4
Formula $C_{14}H_{20}N_3O_3{*}BF_4$
Mol. weight 278,33*86,80 g/mol



RL-1159 TOTT

S-(1-Oxo-2-pyridyl)-thio-N,*N*,*N*',*N*'-tetramethyluronium tetrafluoroborate

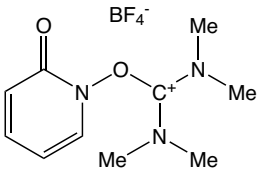
CAS-No.	255825-38-8
Formula	$C_{10}H_{16}N_3OS*BF_4$
Mol. weight	226,32*86,80 g/mol



RL-1064 TPTU

O-(1,2-Dihydro-2-oxo-pyridyl]-N,*N*,*N*',*N*'-tetramethyluronium tetrafluoroborate

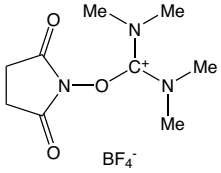
CAS-No.	125700-71-2
Formula	$C_{10}H_{16}N_3O_2*BF_4$
Mol. weight	210,25*86,80 g/mol



RL-1067 TSTU

N,*N*,*N*',*N*'-Tetramethyl-O-(succinimidyl)uronium tetrafluoroborate

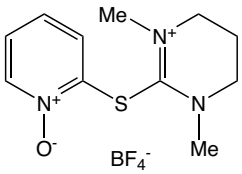
CAS-No.	105832-38-0
Formula	$C_9H_{16}N_3O_3*BF_4$
Mol. weight	214,24*86,80 g/mol



RL-1161 TPTDP

S-(1-Oxo-2-pyridyl)-thio-1,3-dimethylpropyleneuronium tetrafluoroborate

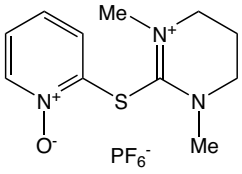
CAS-No.	367252-09-3
Formula	$C_{11}H_{16}N_3OS*BF_4$
Mol. weight	238,33*86,80 g/mol



RL-1158 HPTDP

S-(1-Oxo-2-pyridyl)-thio-1,3-dimethylpropyleneuronium hexafluorophosphate

CAS-No.	366821-62-7
Formula	$C_{11}H_{16}N_3OS*PF_6$
Mol. weight	238,33*144,96 g/mol



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Phosphonium Salts
Uronium and Aminium Salts
Acid-Halides
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5. Acid-Halides

The activation of carboxylic acids *via* carboxylic halides, especially acid chlorides, followed by their reaction with free amines, is one of the most well-established methods for peptide bond formation. The high reactivity of acid chlorides enables rapid and efficient coupling reactions, making them especially useful when working with bulky or less nucleophilic amino acids. However, this reactivity often comes at the expense of chiral integrity, as epimerization might occur under the coupling conditions. In large-scale peptide synthesis, reagents such as thionyl chloride ($SOCl_2$), oxalyl chloride ($(COCl)_2$) and pivaloyl chloride are commonly applied for activation due to their high effectiveness (see *Fig. 19*).

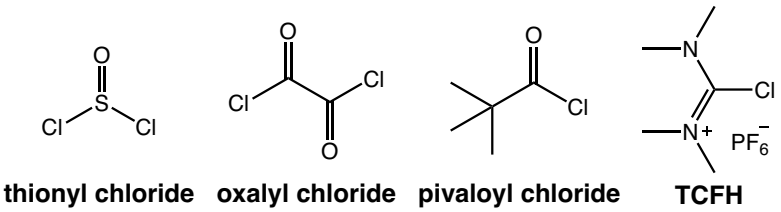

Fig. 19: Commonly used chlorinating reagents used in peptide bond formation; TCFH = tetramethylchloroformamidinium hexafluorophosphate.

Among these, thionyl chloride, particularly when used in pyridine, is one of the most widely utilized chlorinating agents. In the context of Fmoc-protected amino acids, thionyl chloride is typically used in heated dichloromethane (DCM). A notable disadvantage of this approach is the generation of hydrogen chloride which is crucial when using acid-labile protecting groups. This by-product necessitates neutralization with a base, a step that can inadvertently facilitate the formation of oxazolones (see *Fig. 2*) or ketenes (see *Fig. 20*), ultimately resulting in racemization.

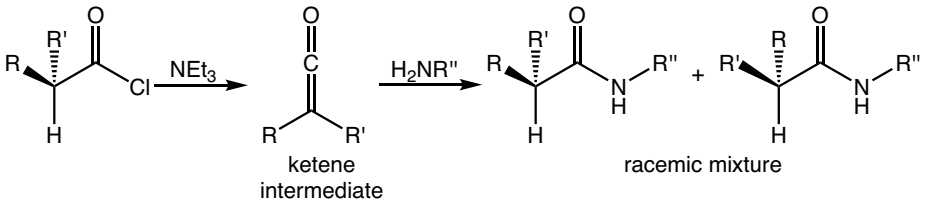

Fig. 20: Formation of a ketene intermediate in basic conditions during neutralization step.

The addition of a small amount of DMF during the formation of acid chlorides with thionyl chloride can serve as a catalyst, forming a Vilsmeier-Haack intermediate (commonly referred to as the Vilsmeier reagent) (see *Fig. 21*).

After activation, the peptide bond formation step is performed under inert conditions.

Note:

Despite its widespread use due to cost-effectiveness, thionyl chloride poses the risk of producing dimethylcarbamoyl chloride, a potentially carcinogenic by-product.

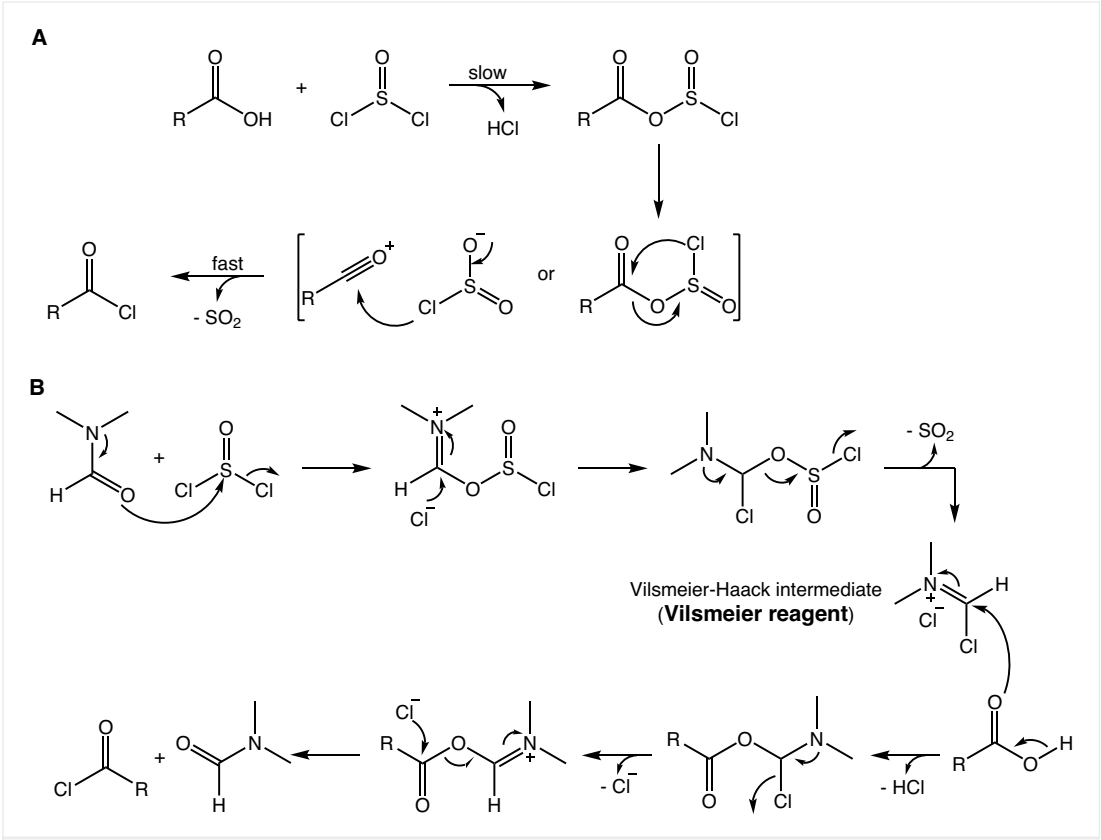

Fig. 21: Thionyl chloride as chlorinating reagent for carboxylic acids, A) uncatalyzed, B) DMF-catalyzed.

Chlor-*N,N,N',N'*-tetramethylformamidinium-hexafluorophosphat (TCFH, *RL-1071 on page 27*) is another chlorinating reagent used as precursor for the synthesis of other coupling reagents such as HATU (*RL-1190 on page 20*) or HCTU (*RL-1031 on page 20*). However, it can also be used directly as a coupling reagent in ACN or water, for example, but also in the synthesis on solid support.

Acid fluorides exhibit slower rates in oxazolone formation in the presence of tertiary amines and provide greater stability against moisture and acid-labile functional groups compared to their chloride counterparts. Fluoroformamidinium salts, such as tetramethylfluoroformamidinium hexafluorophosphate (TFFH, *RL-1077 on page 27*), are highly effective for *in situ* acid fluoride generation.

TFFH's air-stable, non-hygroscopic nature makes it an ideal reagent for peptide synthesis. This method is especially beneficial for amino acids like arginine (Arg) and histidine (His), as their acid fluorides are too unstable for direct isolation or extended storage. Recently, TFFH (*RL-1077 on page 27*) was declared as a green coupling reagent.

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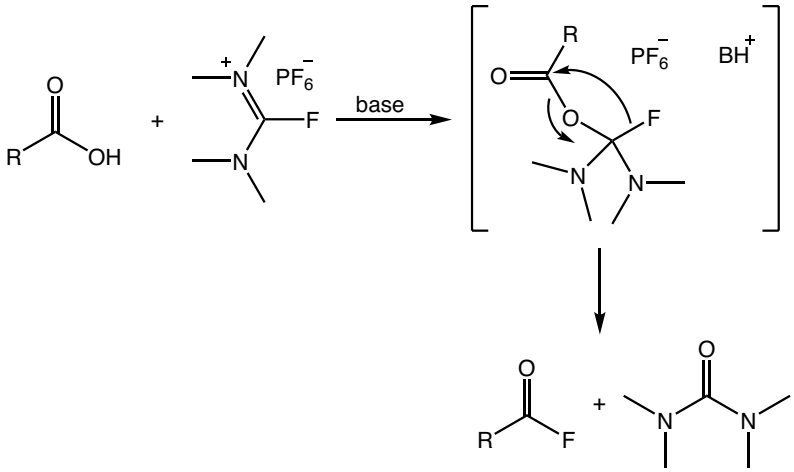

Fig. 22: In situ formation of acid fluorides with TFFH.

Guidelines for coupling procedures

Acyl halides

Coupling reactions are typically conducted in inert dry solvents alongside non-nucleophilic tertiary amines such as NEt_3, DIPEA or NMM. Common organic solvents employed in these reactions include THF, n-heptane, toluene, ACN, or dimethoxyethane (DME). Notably, acyl chlorides exhibit sufficient stability to undergo coupling with amines under aqueous conditions, by utilizing bases like NaOH, $NaHCO_3$, K_2CO_3 under Schotten–Baumann conditions. The reaction kinetics can be further enhanced by the addition of catalytic amounts of pyridine or *N,N*-dimethylaminopyridine (DMAP), with pyridine sometimes used as the solvent.

General procedure

I. In an inert atmosphere dissolve the acyl chloride (2 mmol) in dry THF (4 mL).
II. Cool the solution to 0 °C.
III. Add potassium phosphate (5 mmol) at once and add the amino acid (2 mmol) to the reaction mixture.
IV. Stir the resulting mixture for 12 h at room temperature.
V. The reaction was quenched with water (10 mL) and EtOAc (4 mL).
VI. The organic phase can be discarded, while the pH of the aqueous phase is adjusted to pH = 2.
VII. Extract with EtOAc (2 x 10 mL) and wash with water (6 mL).
VIII. Dry the organic phase with $MgSO_4$ and remove the solvent *in vacuo*.

TCFH (solution phase) (*RL-1071 on page 27*)

I. Dissolve the *N*-protected amino acid (1 eq.), the amino acid component (1.3 eq.), and *N*-methylimidazole (3.5 eq.) in ACN (4 mL).
II. Add TCFH (1.2 eq.) in a single portion.
III. Stir the resulting mixture for 19 h at room temperature.
IV. After completion of the reaction, add isopropyl acetate (6 mL) and H_2O (4 mL) and separate the phases.
V. Extract the aqueous phase with isopropyl acetate (4 mL).
VI. Wash the combined organic phases with water (6 mL).
VII. Dry the organic phase with $MgSO_4$ and remove the solvent *in vacuo*.

TFFH (*RL-1077 on page 27*)

I. In a separate flask or tube, dissolve the *N*-protected amino acid (5 eq. relative to the resin loading) in DMF.

II. Add TFFH (*RL-1077 on page 27*) (5 eq.) and DIPEA (10 eq.) to the solution (total concentration 0.3 M).

III. Pre-activate the resulting mixture for 8-15 min at room temperature. Note: Complete conversion of hindered amino acids such as Aib might take 1-2 h.

IV. Add it to the pre-swelled resin and shake the resin with the coupling mixture for 30 min. Note: For sterically-hindered amino acids (e.g., Aib) use a coupling time of 60 min or repeat the procedure.

V. Remove the excess reagents after the desired reaction time.

VI. Wash the resin with DMF (at least 3 x 5 mL).

References:

- *Peptide Synthesis via Amino Acids Halides, L. A. Carpino, M. Beyermann, H. Wenschuh, M. Biener;* ***Acc. Chem. Res.*** *1996;* ***29(6)****: 268-274.* *https://doi.org/10.1021/ar950023w*
- *Large-scale applications of amide coupling reagents for the synthesis of pharmaceuticals, J. R. Dunetz, J. Magano, G. A. Weisenburger;* ***Org. Process Rev. Des.*** *2016;* ***20(2)****: 140-177.* *https://doi.org/10.1021/op500305s*
- *Amide bond formation and peptide coupling; C. A. G. N. Montalbetti, V. Falque;* ***Tetrahedron*** *2005;* ***61(46)****: 10827-10852.* *https://doi.org/10.1016/j.tet.2005.08.031*
- *Tetramethylfluoroformamidinium Hexafluorophosphate: A Rapid-Acting Peptide Coupling Reagent for Solution and Solid Phase Peptide Synthesis; L. A. Carpino, A. El-Faham;* ***J. Am. Chem. Soc.*** *1995;* ***117(19)****: 5401-5402.* *https://doi.org/10.1021/ja00124a040*
- *TCFH–NMI: Direct Access to N-Acyl Imidazoliums for Challenging Amide Bond Formations; G. L. Beutner, I. S. Young, M. l. L. Davies, M. R. Hickey, H. Park, J. M. Stevens, Q. Ye;* ***Org. Lett.*** *2018;* ***20(14)****: 4218-4222.* *https://doi.org/10.1021/acs.orglett.8b01591*
- *O-Benzotriazolyl-N,N,N',N'-tetramethyluronium Hexafluorophosphate as Coupling Reagent for the Synthesis of Peptides of Biological Interest; V. Dourtoglou, B. Gross, V. Lambropoulou, C. Zioudrou;* ***Synthesis*** *1984;* ***7****: 572-574.* *https://doi.org/10.1055/s-1984-30895*
- *Beyond Amide Bond Formation: TCFH as a Reagent for Esterification; N. R. Luis, K. K. Chung, M. R. Hickey, Z. Lin, G. L. Beutner, D. A. Vosburg;* ***Org. Lett.*** *2024;* ***26(14)****: 2745-2750.* *https://doi.org/10.1021/acs.orglett.3c01611*
- *Progress towards the synthesis of piperazimycin A: synthesis of the non-proteogenic amino acids and elaboration into dipeptides; J. P. Kennedy, C. W. Lindsley;* ***Tetrahedron Letters*** *2010;* ***51(18)****: 2493-2496.* *https://doi.org/10.1016/j.tetlet.2010.02.168*
- *Circular Aqueous Fmoc/t-Bu Solid-Phase Peptide Synthesis; J. Pawlas, J. H. Rasmussen;* ***ChemSusChem*** *2021;* ***14(16)****: 3231-3236.* *https://doi.org/10.1002/cssc.202101028*
- *Fmoc amino acid fluorides in peptide synthesis - Extension of the method to extremely hindered amino acids; H. Wenschuh, M. Beyermann, R.Winter, M. Bienert, D. Ionescu, L. A. Carpino;* ***Tetrahedron Letters*** *1996;* ***37(31)****: 5483-5486.* *https://doi.org/10.1016/0040-4039(96)01160-4*
- *Peptide assembly in the absence of base via Fmoc amino acid fluorides; H. Wenschuh, M. Beyermann, A. El-Faham, S. Ghassemi, L. A. Carpino, M. Bienert;* ***J. Chem. Soc., Chem. Commun.*** *1995;* ***6****: 669-670.* *https://doi.org/10.1039/C39950000669*
- *Eine Methode zur katalysierten Herstellung von Carbonsäure- und Sulfosäure-chloriden mit Thionylchlorid; H. H. Bosshard, R. Mory, M. Schmid, Hch. Zollinger;* ***Helvetica*** *1959;* ***42(5)****: 1653-1658.* *https://doi.org/10.1002/hlca.19590420526*
- *Sustainability Challenges in Peptide Synthesis and Purification: From R&D to Production; A. Isidro-Llobet, M. N. Kenworthy, S. Mukherjee, M. E. Kopach, K. Wegner, F. Gallou, A. G. Smith, F. Roschangar;* ***J. Org. Chem.*** *2019;* ***84(8)****: 4615-4628.* *https://doi.org/10.1021/acs.joc.8b03001*
- *Utilization of N,N,N',N'-Tetramethylfluoroformamidinium Hexafluorophosphate (TFFH) in Peptide and Organic Synthesis; A. El-Faham, S. N. Khattab;* ***Synlett*** *2006;* ***6****:886-904.* *https://doi.org/10.1055/s-0028-1088211*
- *Design and Synthesis of New Immonium-Type Coupling Reagents; A. El-Faham, S. N. Khattab, M. Abdul-Ghani, F. Albericio;* ***Eur. J. Org. Chem.*** *2006;* ***6****: 1563-1573.* *https://doi.org/10.1002/ejoc.200500630*
- *An improved method of amide synthesis using acyl chlorides; L. Zhang, X.-J. Wang, J. Wang, N. Grinberg, D. K. Krishnamurthy, C.H. Senanayake;* ***Tetrahedron Letters*** *2009;* ***50(24)****: 2964-2966.* *https://doi.org/10.1016/j.tetlet.2009.03.220*

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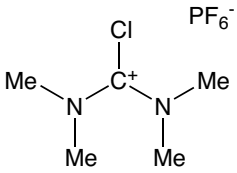
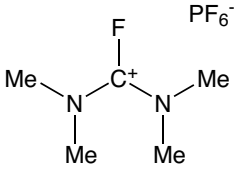
RL-1071 TCFH N,*N*,*N*',*N*'-Tetramethylchloroformamidinium-hexafluorophosphate CAS-No. 207915-99-9 Formula $C_5H_{12}ClN_2{}^*PF_6$ Mol. weight 135,61*144,96 g/mol 	
RL-1077 TFFH N,*N*,*N*',*N*'-Tetramethylfluoroformamidinium hexafluorophosphate CAS-No. 164298-23-1 Formula $C_5H_{12}FN_2{}^*PF_6$ Mol. weight 119,16*144,96 g/mol 	

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6. Anhydrides

Peptide bond formation by anhydrides is one of the oldest and most established methods in peptide chemistry. Anhydrides can be classified into symmetrical, mixed, and cyclic types (see *Fig. 23*), each offering distinct properties and applications in peptide synthesis.

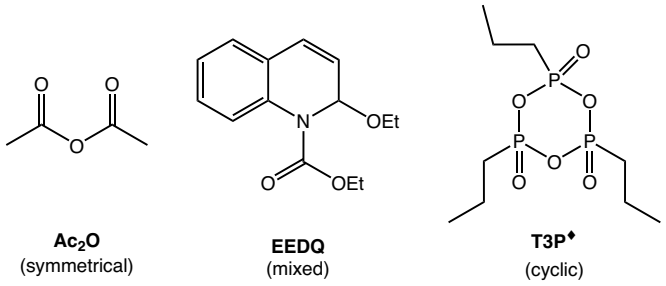

Fig. 23: Selected anhydrides used in peptide synthesis. Ac2O = Acetanhydride; EEDQ = 2-ethoxy-1ethylcarbonyl-1,2-dihydoquinoline; T3P® = n-propylphosphonic anhydride.

In recent years, n-propylphosphonic anhydride (T3P®) has become increasingly popular, especially for large/industrial-scale processes peptide synthesis. Like TFFH (*RL-1077 on page 27*) (chapter 5), T3P® is recognized as an environmentally friendly ("green") coupling reagent. It is widely used in solution phase synthesis for amide bond formation. T3P® offers several notable advantages, including low toxicity, long shelf-life, and an easy handling.

A key advantage of T3P® is that it produces water-soluble side products, which can be easily removed by extraction into the aqueous phase, greatly simplifying purification (see *Fig. 24*). Additionally, T3P® induces minimal epimerization, making it ideal for synthesizing peptides with sensitive α-stereocenters

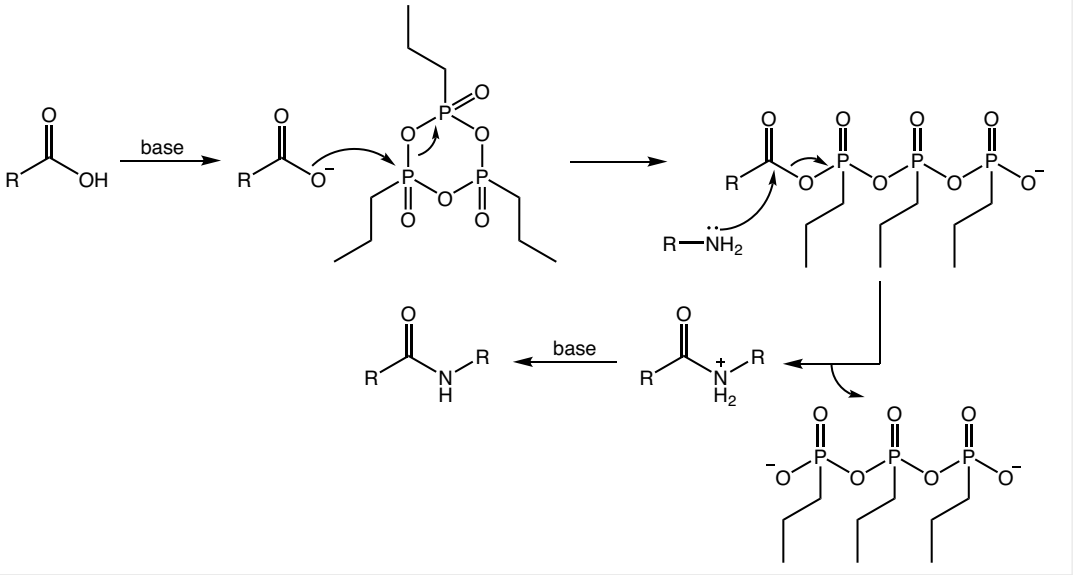

Fig. 24: Coupling mechanism of T3P®.

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Guidelines for coupling with T3P®

I. In a separate flask or tube, dissolve the *N*-protected amino acid (5 eq. relative to the resin loading) in DMF (1 mL/100 mg of resin) and sonicate for 10 min.
II. Add DIPEA (10 eq.) and add T3P® (5 eq.).
III. Add the resulting mixture to the pre-swelled resin.
IV. Add OxymaPure® (5 eq.).
V. Shake the resin with the coupling mixture for 60 min at 60 °C.
VI. Remove the excess reagents after the desired reaction time.
VII. Wash the resin with DMF (at least 3 x 5 mL).
VIII. Repeat the procedure in case of sterically-hindered amino acids.

References:

→ *Large-Scale Amidations in Process Chemistry: Practical Considerations for Reagent Selection and Reaction Execution; J. Magano;* ***Org. Process Res. Dev.*** *2022;* ***26(6)****: 1562-1689.* https://doi.org/10.1021/acs.oprd.2c00005
→ *Propylphosphonic Anhydride (T3P®) as Coupling Reagent for Solid-Phase Peptide Synthesis; O. Al Musaimi, R. Wisdom, P. Talbiersky, B.G. De La Torre, F. Albericio;* ***ChemistrySelect*** *2021;* ***6(11)****: 2649-2657.* https://doi.org/10.1002/slct.202100123
→ *The use of microwave radiations and propylphosphonic anhydride (T3P®) for rapid and highly yielding amide bond formation and peptide coupling; E. Rouge, J. M. Brunel;* ***Tetrahedron*** *2024;* ***151****: 133813.* https://doi.org/10.1016/j.tet.2023.133813
→ *Speeding up sustainable solution-phase peptide synthesis using T3P® as a green coupling reagent: methods and challenges; A. Mattellone, D. Corbisiero, L. Ferrazzano, P. Cantelmi, G. Martelli, C. Palladino, A. Tolomelli, W. Cabri;* ***Green Chem.*** *2023;* ***25****: 2563-2571.* https://doi.org/10.1039/D3GC00431G

7. Triazines

Triazines, for example derivatives such as 2,4,6-trichloro-1,3,5-triazine (cyanuric chloride) and 2-chloro-4,6-dimethoxy-1,3,5-triazine (CDMT), are widely utilized as efficient coupling reagents in peptide bond formation. These heterocyclic compounds (see *Fig. 25*), consisting of a six-membered ring structure with alternating carbon and nitrogen atoms, serve to activate carboxyl groups, facilitating amide bond formation by promoting the nucleophilic attack by amines.

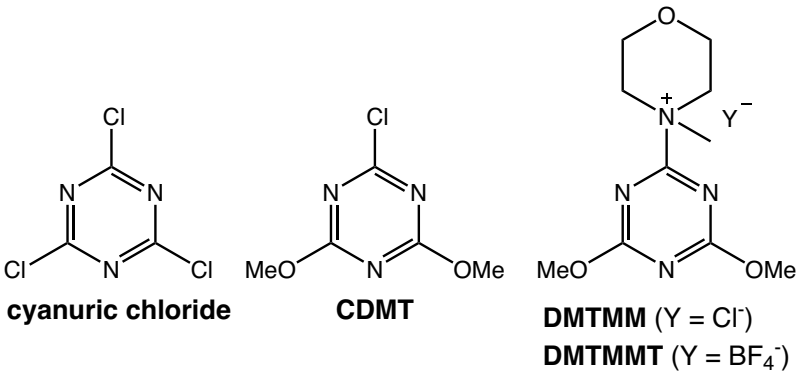

Fig. 25: Chosen triazine coupling reagents.

Cyanuric chloride plays a key role in the synthesis of acyl chlorides, amides, and peptides, acting as a versatile platform for derivatization. Its derivative, CDMT is known for forming highly reactive esters with carboxylic acids, making it an effective acylating agent for amines and, to a lesser extent, alcohols. This activation is typically achieved in the presence of a base, such as NMM, which enhances the reactivity of CDMT. The reaction between NMM and CDMT produces the intermediate 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholin-1-ium chloride (DMTMM), which can be isolated and utilized independently. This feature eliminates the need for prior activation of carboxylic acids, simplifying the synthesis process.

DMTMM has emerged as an efficient coupling agent in peptide synthesis, offering high product yields in one-step reactions under mild conditions (room temperature and atmospheric pressure). Notably, it exhibits compatibility with a range of easily removable solvents such as methanol, ethanol, isopropanol, and water, eliminating the need for rigorous solvent drying procedures. The reaction proceeds without requiring additional additives, and carboxylic acids can be activated *in situ*, broadening its utility in various synthetic applications. Furthermore, DMTMM has demonstrated comparable coupling efficiency to commonly used peptide synthesis reagents like PyBOP (*RL-2005 on page 15*), while minimizing racemization to undetectable levels during solid-phase peptide synthesis.

The reaction mechanism of DMTMM is based on nucleophilic aromatic substitution (S_NAr). In this mechanism, the activated ester intermediate is displaced by the amine, leading to amide bond formation (see *Fig. 26*). NMM is simultaneously regenerated as a by-product, eliminating the need for an additional base in the reaction. The secondary by-product, triazinone, can be easily removed by aqueous washing, simplifying post-reaction purification.

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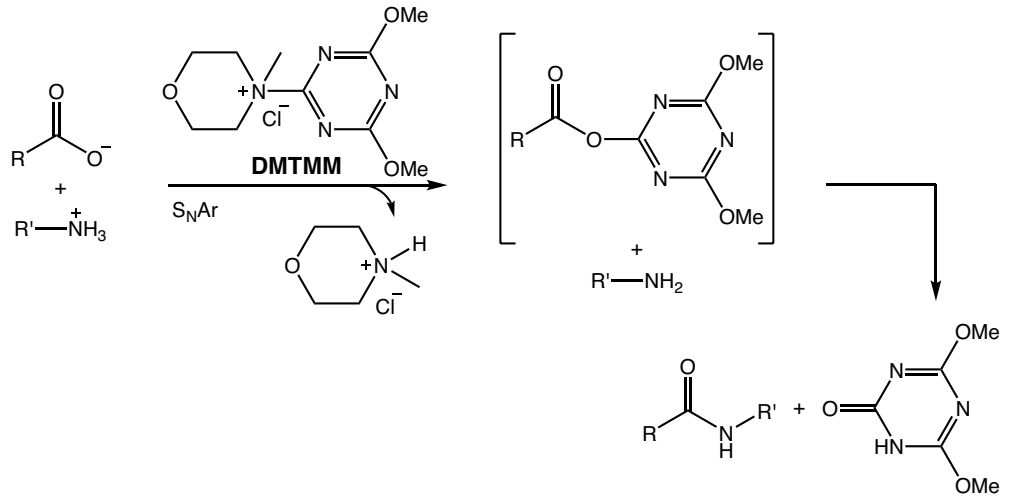


Fig. 26: Coupling mechanism with DMTMM.

However, a notable limitation of this reaction is the release of one equivalent of NMM.

Various studies have explored alternative tertiary amines to replace NMM, evaluating their reactivity alongside DMTMM-like reagents.

Guidelines for coupling with DMTMMT

I. In a separate flask or tube, dissolve the *N*-protected amino acid (3 eq. relative to the resin loading) in DMF (5 mL/g of resin).

II. Add DMTMMT (*RL-1105 on page 32*) (3 eq.) and NMM (6 eq.).

III. Add the resulting mixture to the pre-swelled resin.

IV. Shake the resin with the coupling mixture for 15 min (perform Kaiser-test).

V. Remove the excess reagents after the desired reaction time.

VI. Wash the resin with DMF and DCM (2 x 5 mL for 2 min).

References:

→ *Triazine-based condensing reagents; Z. J. Kamiński;* ***Pept. Sci.*** *2000;* ***55(2)****:140-164. https://doi.org/10.1002/1097-0282(2000)55:2<140::AID-BIP40>3.0.CO;2-B*

→ *N-Triazinylammonium Tetrafluoroborates. A New Generation of Efficient Coupling Reagents Useful for Peptide Synthesis; Z. J. Kamiński, B. Kolesińska, J. Kolesińska, G. Sabatino, M. Chelli, P. Rovero, M.Błaszczyk, M. L. Główka, A. M. Papini;* ***J. Am. Chem. Soc.*** *2005;* ***127(48)****: 16912-16920. https://doi.org/10.1021/ja054260y*

→ *Formation of carboxamides by direct condensation of carboxylic acids and amines in alcohols using a new alcohol- and water-soluble condensing agent: DMT-MM; M. Kunishima, C. Kawachi, K. Hioki, K. Terao, S. Tani;* ***Tetrahedron*** *2001;* ***57(8)****: 1551-1558. https://doi.org/10.1016/S0040-4020(00)01137-6*

→ *4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methyl-morpholinium chloride: an efficient condensing agent leading to the formation of amides and esters; M. Kunishima, C. Kawachi, J. Monta, K. Terao, F. Iwasaki, S. Tani;* ***Tetrahedron*** *1999;* ***55(46)****: 13159-13170. https://doi.org/10.1016/S0040-4020(99)00809-1*

→ *Synthesis of sterically-hindered peptidomimetics using 4-(4,6-dimethoxy-1,3,5-triazine-2-yl)-4-methyl-morpholinium chloride; W.-C. Shieh, Z. Chen, S. Xue, J. McKenna, R.-M. Wang, K. Prasad, O. Repič;* ***Tetrahedron Letters*** *2008;* ***49(37)****: 5359-5362. https://doi.org/10.1016/j.tetlet.2008.06.119*

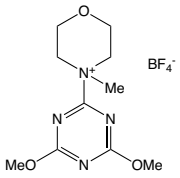
- *4-(4,6-Dimethoxy[1,3,5]triazin-2-yl)-4-methyl-morpholinium Chloride (DMTMM): A Valuable Alternative to PyBOP for Solid Phase Peptide Synthesis; A. Falchi, G. Giacomelli, A. Porcheddu, M. Taddei;* ***Synlett*** *2000;* ***2****: 275-277.* https://doi.org/10.1055/s-2000-6490
- *Aqueous microwave-assisted solid-phase peptide synthesis using Fmoc strategy. III: Racemization studies and water-based synthesis of histidine-containing peptides; K. Hojo, N. Shinozaki, K. Hidaka, Y. Tsuda, Y. Fukumori, H. Ichikawa, J. D. Wade;* ***Amino Acids*** *2014;* ***46****: 2347-2354.* https://doi.org/10.1007/s00726-014-1779-y
- *A systematic analysis of DMTMM vs EDC/NHS for ligation of amines to hyaluronan in water; M. D'Este, D. Eglin, M. Alini;* ***Carbohydr Polym*** *2014;* ***108****: 239-246.* https://doi.org/10.1016/j.carbpol.2014.02.070
- *Recent development in peptide coupling reagents; T. I. Al-Warhi, H. M. A. Al-Hazimi, A. El-Faham;* ***Journal of Saudi Chemical Society*** *2012;* ***16(2)****: 97-116* https://doi.org/10.1016/j.jscs.2010.12.006

Product details

RL-1105 DMTMMT

4-(4,6-Dimethoxy-1,3,5-triazin-2-yl)-4-methyl-morpholinium tetrafluoroborate

CAS-No.	293311-03-2
Formula	$C_{10}H_{17}BF_4N_4O_3$
Mol. weight	241,27*86,80 g/mol



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8. Ynamides

Ynamides are novel coupling reagents characterized by a triple bond to a nitrogen atom that is substituted with an electron-withdrawing group (see *Fig. 27*).

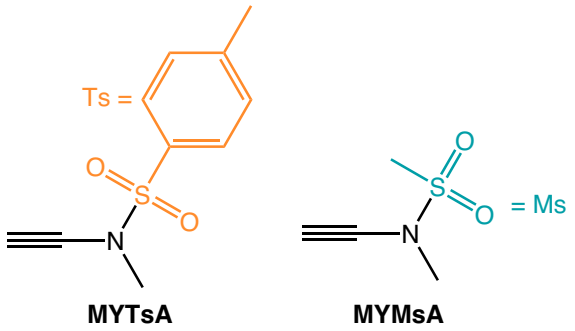

Fig. 27: Prominent ynamide coupling reagents.

These compounds can be utilized in a two-step, one-pot methodology to facilitate the formation of peptide bonds. This process involves the efficient hydroacylation of ynamides with carboxylic acids, followed by aminolysis (see *Fig. 28*). Notably, these reactions occur under mild conditions, allowing for the exclusion of a base, which prevents the deprotonation of the Cα atom and avoids the formation of oxazolones, thereby eliminating the risk of racemization.

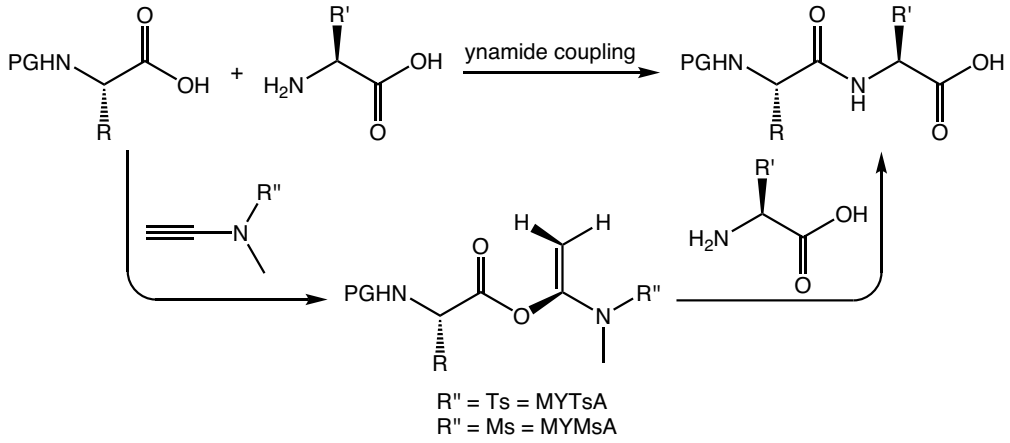

Fig. 28: Two-step, one-pot procedure of ynamide coupling.

Furthermore, ynamides exhibit stability under both air and moisture and can be applied without the need for additives or catalysts, rendering them suitable for peptide segment condensations and large-scale synthesis. Additionally, ynamides demonstrate compatibility with various functional groups found in side-chains of amino acids, including hydroxyl groups (serine, threonine), thiols (cysteine), amides (asparagine, glutamine), and the amine functionality of indole (tryptophane).

MYTsA (*RL-8665 on page 35*) offers an additional advantage by enabling peptide synthesis in the N → C direction, mirroring natural protein biosynthesis (see *Fig. 29*). This method avoids the issues of racemization associated with carbodiimides and offers a superior atom economy, resulting in less waste. Furthermore, the amino groups of the amino acid building blocks do not require protection or deprotection in this process, thereby eliminating the need for Fmoc protecting group and base (commonly piperidine).

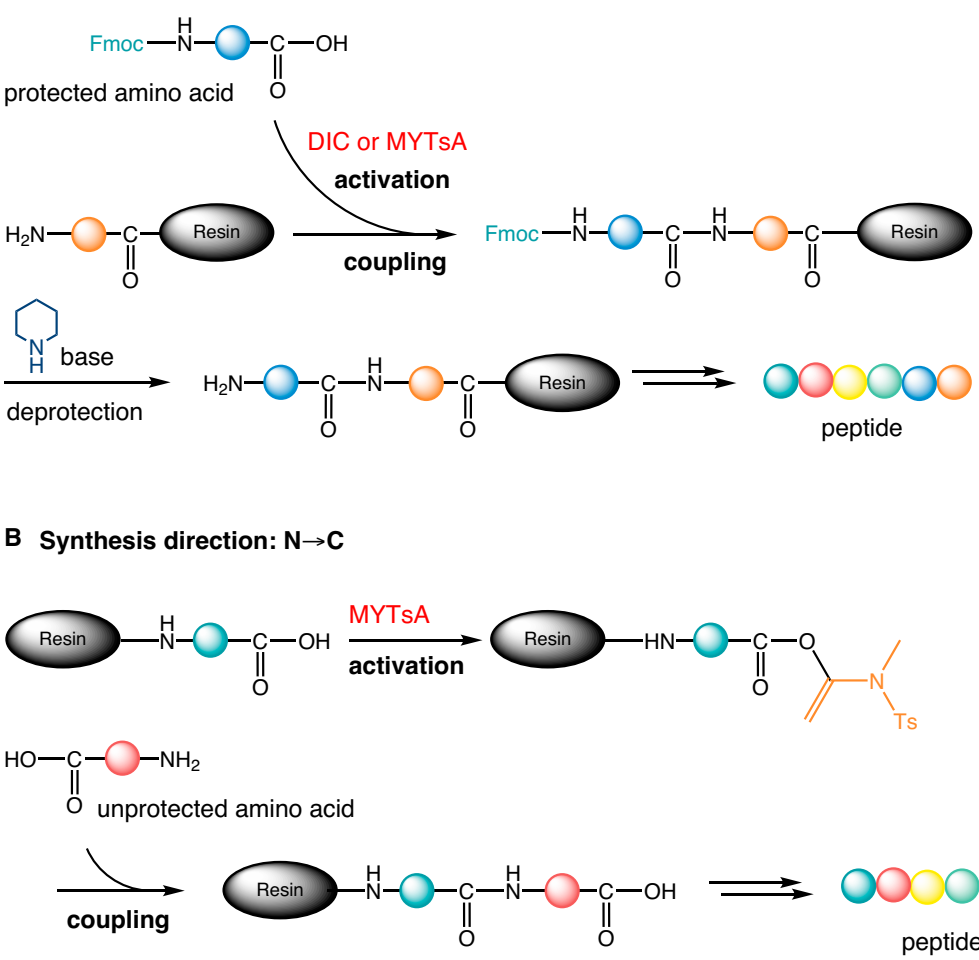

Fig. 29: Conventional vs. inverse peptide synthesis on solid support.

Coupling guidelines

I. Dissolve MYTsA (0.2 mmol) and the *N*-protected amino acid (0.2 mmol) in DCM (1 mL).
II. Stir the resulting mixture until full consumption of MYTsA (TLC control).
III. Add the amino acid (0.2 mmol) to the formed α-acyloxyenamide.
IV. Stir the reaction mixture until full consumption of α-acyloxyenamide (TLC control).
V. Concentrate the reaction mixture *in vacuo* and purify the crude product by silica gel chromatography.

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References:

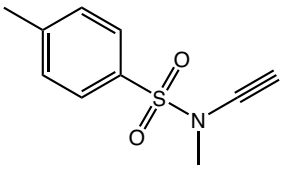
- *Ynamides as Racemization-Free Coupling Reagents for Amide and Peptide Synthesis; L. Hu, S. Xu, Z. Zhao, Y. Yang, Z. Peng, M. Yang, C. Wang, J. Zhao;* ***J. Am. Chem. Soc.*** *2016;* ***138(40)****: 13135-13138. https://doi.org/10.1021/jacs.6b07230*
- *Ynamide: A New Coupling Reagent for Amide and Peptide Synthesis;* ***L. Hu, J. Zhao, Synlett*** *2017;* ***28(14)****: 1663-1670. https://doi.org/10.1055/s-0036-1588860*
- *Ynamide Coupling Reagents: Origin and Advances; L. Hu, J. Zhao;* ***Acc. Chem. Res.*** *2024;* ***57(6)****: 855-869. https://doi.org/10.1021/acs.accounts.3c00743*
- *Ynamide Coupling Reagent for the Chemical Cross-Linking of Proteins in Live Cells; S. Li, C. Zhu, Q. Zhao, Z. M. Zhang, P. Sun, Z. Li;* ***ACS Chem Biol.*** *2023;* ***18(6)****: 1405-1415. https://doi.org/10.1021/acschembio.3c00149*
- *Ynamides: A Modern Functional Group for the New Millennium; K. A. DeKorver, H. Li, A. G. Lohse, R. Hayashi, Z. Lu, Y. Zhang, R. P. Hsung;* ***Chem. Rev.*** *2010;* ***110(9)****: 5064-5106. https://doi.org/10.1021/cr100003s*
- *Exploiting Remarkable Reactivities of Ynamides: Opportunities in Designing Catalytic Enantioselective Reactions; J. Luo, G.-S. Chen, S.-J. Chen, J.-S. Yu, Z.-D. Li, Y. L. Liu;* ***ACS Catal.*** *2020;* ***10(23)****: 13978-13992. https://doi.org/10.1021/acscatal.0c04180*
- *Recent advances in asymmetric synthesis of chiral amides and peptides: racemization-free coupling reagents; Y. Guo, M. Wang, Y. Gao, G. Liu;* ***Org. Biomol. Chem.*** *2024;* ***22(22)****: 4420-4425. https://doi.org/10.1039/D4OB00563E*
- *Ynamide-Mediated Peptide Bond Formation: Mechanistic Study and Synthetic Applications; S. Xu, D. Jiang, Z. Peng, L. Hu, T. Liu, L. Zhao, J. Zhao;* ***Angew. Chem. Int. Ed.*** *2022;* ***61(46)****: e202212247. https://doi.org/10.1002/anie.202212247*
- *Inverse Peptide Synthesis Using Transient Protected Amino Acids; T. Liu, Z. Peng, M. Lai, L. Hu, J. Zhao;* ***J. Am. Chem. Soc.*** *2024;* ***146(6)****: 4270-4280. https://doi.org/10.1021/jacs.4c00314*
- *Water-removable ynamide coupling reagent for racemization-free syntheses of peptides, amides, and esters; T. Liu, X. Zhang, Z. Peng, J. Zhao;* ***Green Chem.*** *2021;* ***23(24)****: 9916-9921. https://doi.org/10.1039/D1GC03498G*

Product details

RL-8665 MYTsA

N-ethynyl-N,4-dimethylbenzenesulfonamide

CAS-No.	1005500-75-3
Formula	$C_{10}H_{11}NO_2S$
Mol. weight	209,26 g/mol



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In 2001 Iris Biotech GmbH was founded just at the beginning of the Biotech movement and the first remarkable breakthrough of biotech pharma products. Although the biotech field is rather young compared to other industries we believe on long-term business, a good partnership between our business partners and Iris Biotech GmbH and a good reputation. It is our duty as well as our responsibility to maintain and to extend this over the next generations – based on the principles of an honourable and prudent tradesman which based upon the concept of honourable entrepreneurship.

This Code of Conduct has been developed following the "Voluntary Guidelines for Manufacturers of Fine Chemical Intermediates and Active Ingredients" issued by AIME (Agrochemical & Intermediates Manufacturers in Europe) and the requirements of some of our business associates.

Iris Biotech GmbH commits to hold this Code of Conduct and to include and apply its principles in the management system and the company policies.

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Labour

Iris Biotech GmbH is committed to uphold the human rights of workers and to treat them with dignity and respect. Child labour, workplace harassment, discrimination, and harsh and inhumane treatment are prohibited. Iris Biotech GmbH respects the rights of the employees to associate freely, join or not join labour unions, seek representation and join workers' councils. Employees are paid and their working timetable is established according to applicable wage and labour laws. Employees are able to communicate openly with management regarding working conditions without threat of reprisal, intimidation or harassment.

General Policies

Contracts and Secrecy Agreements are binding and the confidential information received is only used for intended purposes. Clear management and organizational structures exist to provide efficient normal working and to address problems quickly. Know-how is protected and intellectual property isrespected.

Health and Safety

Iris Biotech GmbH provides a safe and healthy working environment to the employees and protects them from overexposure to chemical and physical hazards. Products are produced, stored and shipped under the guidelines of the relevant chemical and safety legislation. Risks and emergency scenarios are identified and evaluated, and their possible impact is minimized by implementing emergency plans and written procedures. Safety information regarding hazardous materials is available to educate, train and protect workers from hazards. Preventive equipment and facilities maintenance is performed at suitable periods to reduce potential hazards. Employees are regularly trained in health and safety matters and are informed about product properties and risk classification when it is required.

Environment

Iris Biotech GmbH operates in an environmentally responsible and efficient manner, minimizing adverse impacts on the environment. Waste streams are managed to ensure a safe handling, movement, storage, recycling and reuse, before and after being generated. Systems to prevent and mitigate accidental spills and releases to the environment are in place. All required environmental permits and licenses are obtained and their operational and reporting requirements are complied with.

Production and Quality Management

A quality management system following the Good Distribution Practices (GDP rules) of Active Pharmaceutical Ingredients is established covering all the aspects of the worldwide distribution of products. Regular audits are performed to evaluate the efficiency and fulfilling of the quality system. Process controls to provide reproducible product quality are established. There are preventive maintenance procedures to ensure plant reliability and the lowest risk of failure. Staff is trained periodically about GMP and GDP rules. Procedures are established and installations are designed to avoid cross contamination. Batch and analytical records are kept for inspection and audit purposes for suitable periods according guidelines.

Research and Development

Research and development staff education is appropriate to their functional activity and they are trained to develop, optimize and scale-up the processes. Intellectual property is respected and know-how protected. Development of manufacturing processes reflects the principles of the Green Chemistry according to the American Chemical Society Green Chemistry Institute. Animal testing is not used unless alternatives are not scientifically valid or accepted by regulators. If animal testing is carried out, animals are treated so that pain and stress are minimized.

Terms and Conditions of Sales

All orders placed by a buyer are accepted and all contracts are made subject to the terms which shall prevail and be effective notwithstanding any variations or additions contained in any order or other document submitted by the buyer. No modification of these terms shall be binding upon Iris Biotech GmbH unless made in writing by an authorised representative of Iris Biotech GmbH.

Placing of Orders

Every order made by the buyer shall be deemed an offer by the buyer to purchase products from Iris Biotech GmbH and will not be binding on Iris Biotech GmbH until a duly authorised representative of Iris Biotech GmbH has accepted the offer made by the buyer. Iris Biotech GmbH may accept orders from commercial, educational or government organisations, but not from private individuals and Iris Biotech GmbH reserves the right to insist on a written order and/or references from the buyer before proceeding.

There is no minimum order value. At the time of acceptance of an order Iris Biotech GmbH will either arrange prompt despatch from stock or the manufacture/acquisition of material to satisfy the order. In the event of the latter Iris Biotech GmbH will indicate an estimated delivery date. In addition to all its other rights Iris Biotech GmbH reserves the right to refuse the subsequent cancellation of the order if Iris Biotech GmbH expects to deliver theproduct on or prior to the estimated delivery date. Time shall not be of the essence in respect of delivery of the products. If Iris Biotech GmbH is unable to deliver any products by reason of any circumstances beyond its reasonable control („Force Majeure“) then the period for delivery shall be extended by the time lost due to such Force Majeure. Details of Force Majeure will be forwarded by Iris Biotech GmbH to the buyer as soon as reasonably practicable.

Prices, Quotations and Payments

Prices are subject to change. For the avoidance of doubt, the price advised by Iris Biotech GmbH at the time of the buyer placing the order shall supersede any previous price indications. The buyer must contact the local office of Iris Biotech GmbH before ordering if further information is required. Unless otherwise agreed by the buyer and Iris Biotech GmbH, the price shall be for delivery ex-works. In the event that the buyer requires delivery of the products otherwise than ex-works the buyer should contact the local office of Iris Biotech GmbH in order to detail its requirements. Iris Biotech GmbH shall, at its discretion, arrange the buyer‘s delivery requirements including, without limitation, transit insurance, the mode of transit (Iris Biotech GmbH reserves the right to vary the mode of transit if any regulations or other relevant considerations so require) and any special packaging requirements (including cylinders). For the avoidance of doubt all costs of delivery and packaging in accordance with the buyer‘s requests over and above that of delivery in standard packaging ex-works shall be for the buyer‘s account unless otherwise agreed by both parties. Incoterms 2020 shall apply. Any tax, duty or charge imposed by governmental authority or otherwise and any other applicable taxes, duties or charges shall be for the buyer‘s account. Iris Biotech GmbH may, on request and where possible, provide quotations for multiple packs or bulk quantities, and non-listed items. Irrespective of the type of request or means of response all quotations must be accepted by the buyer without condition and in writing before an order will be accepted by Iris Biotech GmbH. Unless agreed in writing on different terms, quotations are valid for 30 days from the date thereof. Payment terms are net 30 days from invoice date unless otherwise agreed in writing. Iris Biotech GmbH reserves the right to request advance payment at its discretion. For overseas transactions the buyer shall pay all the banking charges of Iris Biotech GmbH. The buyer shall not

be entitled to withhold or set-off payment for the products for any reason whatsoever. Government/ Corporate Visa and MasterCard (and other such credit cards) may be accepted on approved accounts for payment of the products. Personal credit cards are not acceptable. Failure to comply with the terms of payment of Iris Biotech GmbH shall constitute default without reminder. In these circumstances Iris Biotech GmbH may (without prejudice to any other of its rights under these terms) charge interest to accrue on a daily basis at the rate of 2% per month from the date upon which payment falls due to the actual date of payment (such interest shall be paid monthly). If the buyer shall fail to fulfil the payment terms in respect of any invoice of Iris Biotech GmbH Iris Biotech GmbH may demand payment of all outstanding balances from the buyer whether due or not and/or cancel all outstanding orders and/or decline to make further deliveries or provision of services except upon receipt of cash or satisfactory securities. Until payment by the buyer in full of the price and any other monies due to Iris Biotech GmbH in respect of all other products or services supplied or agreed to be supplied by Iris Biotech GmbH to the buyer (including but without limitation any costs of delivery) the property in the products shall remain vested in Iris Biotech GmbH.

Shipping, Packaging and Returns

The buyer shall inspect goods immediately on receipt and inform Iris Biotech GmbH of any shortage or damage within five days. Quality problems must be notified within ten days of receipt. Goods must not be returned without prior written authorisation of Iris Biotech GmbH. Iris Biotech GmbH shall at its sole discretion replace the defective products (or parts thereof) free of charge or refund the price (or proportionate price) to buyer. Opened or damaged containers cannot be returned by the buyer without the written prior agreement of Iris Biotech GmbH. In the case of agreed damaged containers which cannot be so returned, the buyer assumes responsibility for the safe disposal of such containers in accordance with all applicable laws.

Product Quality, Specifications and Technical Information

Products are analysed in the Quality Control laboratories of Iris Biotech GmbH's production partners by methods and procedures which Iris Biotech GmbH considers appropriate. In the event of any dispute concerning reported discrepancies arising from the buyer's analytical results, determined by the buyer's own analytical procedures, Iris Biotech GmbH reserves the right to rely on the results of own analytical methods of Iris Biotech GmbH. Certificates of Analysis or Certificates of Conformity are available at the discretion of Iris Biotech GmbH for bulk orders but not normally for prepack orders. Iris Biotech GmbH reserves the right to make a charge for such certification. Specifications may change and reasonable variation from any value listed should not form the basis of a dispute. Any supply by Iris Biotech GmbH of bespoke or custom product for a buyer shall be to a specification agreed by both parties in writing. Technical information, provided orally, in writing, or by electronic means by or on behalf of Iris Biotech GmbH, including any descriptions, references, illustrations or diagrams in any catalogue or brochure, is provided for guidance purposes only and is subject to change.

Safety

All chemicals should be handled only by competent, suitably trained persons, familiar with laboratory procedures and potential chemical hazards. The burden of safe use of the products of Iris Biotech GmbH vests in the buyer. The buyer assumes all responsibility for warning his employees, and any persons who might reasonably be expected to come into contact with the products, of all risks to person and property in any way connected with the products and for instructing them in their safe handling and use. The buyer also assumes the responsibility for the safe disposal of all products in accordance with all applicable laws.

Uses, Warranties and Liabilities

All products of Iris Biotech GmbH are intended for laboratory research purposes and unless otherwise stated on product labels, in the catalogue and product information sheet of Iris Biotech GmbH or in other literature furnished to the buyer, are not to be used for any other purposes, including but not limited to use as or as components in drugs for human or animal use, medical devices, cosmetics, food additives, household chemicals, agricultural or horticultural products or pesticides. Iris Biotech GmbH offers no warranty regarding the fitness of any product for a particular purpose and shall not be responsible for any loss or damage whatsoever arising there from. No warranty or representation is given by Iris Biotech GmbH that the products do not infringe any letters patent, trademarks, registered designs or other industrial rights. The buyer further warrants to Iris Biotech GmbH that any use of the products in the United States of America shall not result in the products becoming adulterated or misbranded within the meaning of the Federal Food, Drug and Cosmetic Act (or such equivalent legislation in force in the buyer's jurisdiction) and shall not be materials which may not, under sections 404, 505 or 512 of the Act, be introduced into interstate commerce. The buyer acknowledges that, since the products of Iris Biotech GmbH are intended for research purposes, they may not be on the Toxic Substances Control Act 1976 („TSCA") inventory. The buyer warrants that it shall ensure that the products are approved for use under the TSCA (or such other equivalent legislation in force in the buyer's jurisdiction), if applicable. The buyer shall be responsible for complying with any legislation or regulations governing the use of the products and their importation into the country of destination (for the avoidance of doubt to include, without limitation, the TSCA and all its amendments, all EINECS, ELINCS and NONS regulations). If any licence or consent of any government or other authority shall be required for the acquisition, carriage or use of the products by the buyer the buyer shall obtain the same at its own expense and if necessary produce evidence of the same to Iris Biotech GmbH on demand. Failure to do so shall not entitle the buyer to withhold or delay payment. Any additional expenses or charges incurred by Iris Biotech GmbH resulting from such failure shall be for the buyer's account. Save for death or personal injury caused by negligence of Iris Biotech GmbH, sole obligation of Iris Biotech GmbH and buyer's exclusive remedy with respect to the products proved to the satisfaction of Iris Biotech GmbH to be defective or products incorrectly supplied shall be to accept the return of said products to Iris Biotech GmbH for refund of the actual purchase price paid by the buyer (or proportionate part thereof), or replacement of the defective product (or part thereof) with alternative product. Iris Biotech GmbH shall have no liability to the buyer under or arising directly or indirectly out of or otherwise in connection with the supply of products by Iris Biotech GmbH to the buyer and/or their re-sale or use by the buyer or for any product, process or services of the buyer which in any way comprises the product in contract tort (including negligence or breach of statutory duty) or otherwise for pure economic loss, loss of profit, business, reputation, depletion of brand, contracts, revenues or anticipated savings or for any special indirect or consequential damage or loss of any nature except as may otherwise be expressly provided for in these terms. All implied warranties, terms and representations in respect of the products (whether implied by statute or otherwise) are excluded to the fullest extent permitted by law. The buyer shall indemnify Iris Biotech GmbH for and against any and all losses, damages and expenses, including legal fees and other costs of defending any action, that Iris Biotech GmbH may sustain or incur as a result of any act or omission by the buyer, its officers, agents or employees, its successors or assignees, its customers or all other third parties, whether direct or indirect, in connection with the use of any product. For the avoidance of doubt and in the event that Iris Biotech GmbH supplies bespoke or custom product to the buyer's design or specification, this indemnity shall extend to include any claim by a third party that the manufacture of the product for the buyer or the use of the product by the buyer infringes the intellectual property rights of any third party.

General

Iris Biotech GmbH shall be entitled to assign or sub-contract all or any of its rights and obligations hereunder. The buyer shall not be entitled to assign, transfer, sub-contract or otherwise delegate any of its rights or obligations hereunder. Any delay or forbearance by Iris Biotech GmbH in exercising any right or remedy under these terms shall not constitute a waiver of such right or remedy. If any provision of these terms is held by any competent authority to be invalid or unenforceable in whole or in part the validity of the other provisions of these terms and the remainder of the provision in question shall not be affected. These terms shall be governed by German Law and the German Courts shall have exclusive jurisdiction for the hearing of any dispute between the parties save in relation to enforcement where the jurisdiction of the German Courts shall be non-exclusive.

Get in Contact

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Distribution Partners

The list contains the current distributors of Iris Biotech in different regions of the world. The latest list of distribution partners and contact details is available at: *www.iris-biotech.de/distribution-partner*

China:

Chengdu Yoo Technology Co., Ltd.

Japan:

BizCom Japan, Inc.

Shigematsu & Co., Ltd

Cosmo Bio Co., Ltd.

USA & Canada:

Peptide Solutions LLC

India, Bangladesh, Oman, Sri Lanka, United Arab Emirates:

Sumit Biosciences Pvt Ltd.

Empowering Peptide Innovation