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COMMONLY USED COUPLING REAGENTS IN PEPTIDE SYNTHESIS

DILUNBIO PEPTIDE SYNTHESIZER

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Commonly Used Coupling Reagents in Peptide Synthesis

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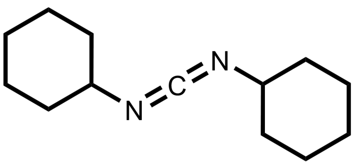
Peptides have emerged as pivotal bioactive molecules in pharmaceutical development, disease diagnosis, materials science, nutraceuticals, and cosmetics due to their high bioactivity, low toxicity, and strong target specificity. These advantages have made peptide synthesis technology a persistent research focus in chemical biology.Since the first artificial synthesis of a dipeptide by Fischer's team in 1901, the field of peptide synthesis has undergone over a century of technological evolution. With peptides gaining increasing strategic importance in innovative drug development and functional material construction among fine chemicals research, their synthetic strategies have achieved groundbreaking progress.

The dominant strategy in contemporary peptide synthesis still relies on directed acylation of natural α-amino acids mediated by coupling reagents. This process enables efficient peptide bond formation through precisely catalyzed intermolecular dehydration between carboxyl and amino groups. To date, researchers have designed and developed over a hundred structurally diverse amide coupling reagents, significantly advancing the development of peptide synthesis. This article summarizes several commonly used coupling reagents in peptide synthesis.

1. Carbodiimide Reagents

1.1 N,N′-Dicyclohexylcarbodiimide (DCC)



DCC

N,N′-Dicyclohexylcarbodiimide (DCC, CAS: 538-75-0, molecular formula: $C_{13}H_{22}N_2$, MW: 206.33) is a bifunctional crosslinking agent. DCC is a commonly used dehydrating agent for esterification and amidation reactions, and is also applicable in the synthesis of anhydrides, aldehydes, ketones, and isocyanates. It was the first coupling reagent employed in peptide synthesis. Despite drawbacks such as generating poorly soluble byproduct Dicyclohexylurea (DCU), which makes it less suitable for solid-phase synthesis, DCC remains widely used in liquid-phase peptide synthesis due to its low cost and easy removal via filtration.

- **Reaction Conditions:**

Temperature: 0-25°C (use ice-water bath to control exotherm)

Time: 2-4 hours (prolong for difficult peptides)

Solvents: DCM, DMF (pre-cool to 0°C)

- **Applicable Scope:**

Linear peptides, Simple cyclic peptides, Non-sterically hindered amino acids (e.g., Gly, Ala, Val)

- **Caution:**

Proline (prone to diketopiperazine byproduct formation)

Avoid unprotected acidic amino acids (Asp, Glu) to prevent side-chain crosslinking

- **Racemization Suppression:**

Add HOBt to suppress racemization

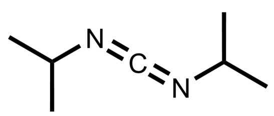
- **Byproduct Handling:**

DCU must be removed by filtration or recrystallization due to its poor solubility in most solvents.

- **Precautions:**

Strong skin irritant – wear gloves in fume hood; hygroscopic – store desiccated

1.2 N,N'-Diisopropylcarbodiimide (DIC)



DIC

N,N'-Diisopropylcarbodiimide (DIC, CAS: 693-13-0, $C_7H_{14}N_2$, MW: 126.20) is a liquid carbodiimide coupling reagent under ambient conditions, offering superior handling convenience compared to solid DCC. As a highly efficient dehydrating agent, DIC catalyzes diverse coupling reactions under mild conditions (e.g., room temperature), including amide bond formation in peptide and nucleic acid synthesis (connecting free carboxyl and amino groups), as well as syntheses of anhydrides, isocyanates, aldehydes, and ketones. This compound serves as a critical dehydrating agent in synthesizing butirosin and glutathione, with extensive applications in pharmaceuticals, biologics, cosmetics, and other fine organic syntheses. Its key advantage lies in the soluble byproduct N,N'-diisopropylurea (DICU) formed post-reaction, which substantially simplifies product isolation and purification.

- **Reaction Conditions**:

Temperature: Room temperature (20–25°C)

Time: 30–120 minutes (extend to 4 hours for sterically hindered substrates)

Solvents: DCM, DMF

Base: N,N-Diisopropylethylamine (DIPEA, 1.5–2.0 equiv, pH 8–9), etc.

- **Applicable Scope**:

Solid-phase synthesis, acid-sensitive protecting group systems (tBu, Trt), Ser/Thr with unprotected side-chain hydroxyl

- **Caution**:

Tryptophan (indole ring oxidation and electrophilic side reactions; suppress with 0.1 M thioglycol)

- **Byproduct Handling**:

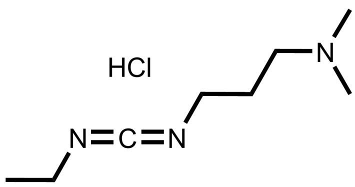
DICU (diisopropylurea) dissolves readily in organic solvents for simplified purification

- **Precautions**:

Strong irritant: Causes dermatitis upon skin contact; corneal damage if eye exposure occurs

Store in cool, ventilated, dry environments

1.3 EDC



EDC

1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC, CAS: 1892-57-5, $C_8H_{17}N_3$•HCl, MW: 191.70) is a water-soluble carbodiimide coupling reagent. It efficiently catalyzes amide bond formation in peptide synthesis by activating carboxyl groups to form O-acylisourea intermediates, and serves as a versatile coupling/crosslinking agent in polysaccharide modification, protein/peptide synthesis, nucleotide conjugation, polymer functionalization, and organic synthesis. Key advantages include operation under non-anhydrous conditions without pre-drying, shortened reaction times, high efficiency, and easy handling. The water-soluble urea byproduct is readily removable by dialysis or extraction. Its mild reaction conditions (pH 4–6 in aqueous media) and excellent biocompatibility make it essential for dehydration condensation and bioconjugation in life sciences.

- **Reaction Conditions:**

Temperature: 0–25°C

pH Control: pH 4.5–5.5 (MES buffer required)

Time: 30 min~2 hours

Solvents: Water/DMF (1:1 v/v)

- **Applicable Scope:**

Protein modification, antibody crosslinking, aqueous-phase reactions (substrates with free amino groups)

Avoid:

High-concentration Cysteine (thiol interference with intermediate stability); Histidine (imidazole ring protonation reduces reactivity in acidic conditions)

- **Racemization Suppression:**

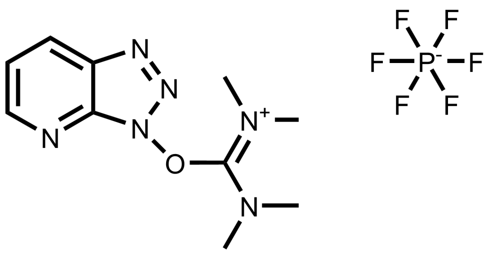
Control NHS reagent equivalents strictly

- **Precautions:**

Pungent odor; weakly acidic nature

2. Uronium Salt Reagents

2.1 HATU



2-(7-Azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (HATU, CAS: 148893-10-1, molecular formula $C_{10}H_{15}F_6N_6OP$, MW: 380.24), is currently the most commonly used uronium-type coupling reagent in peptide synthesis. It effectively overcomes steric hindrance and achieves ideal yields without requiring strictly anhydrous conditions, featuring rapid reaction kinetics and low racemization tendency. HATU outperforms HBTU in peptide nucleic acid (PNA) synthesis.

During synthesis, organic bases such as *N,N*-diisopropylethylamine (DIPEA) or *N*-methylmorpholine (NMM) must be added to activate amino acids. Under alkaline conditions, the carboxyl group on the amino acid dissociates into a carboxylate anion, which attacks HATU to form an O-acyl(tetramethyl)isourea intermediate via an addition-elimination reaction. Subsequently, the 7-azabenzotriazole oxide anion (OAt^-) attacks this intermediate to generate an active ester and tetramethylurea. The amide bond is then formed through base-mediated addition-elimination with the active ester.Although HATU is the most reactive uronium coupling reagent, its high cost may prompt substitution with HBTU or HCTU.

- **Reaction Conditions**:

Temperature: 0–25°C (may increase to 40°C for sterically hindered substrates)

Time: 5–15 minutes (rapid activation)

Solvent: DMF

Base: DIPEA (2.0–3.0 equiv), NMM, etc.

- **Applicable Scope**:

Sterically hindered cyclic structures (e.g., Pro, Aib [α,α-disubstituted hindered residues]), cyclic peptides, bulky amino acids (e.g., Val), and racemization-prone amino acids (e.g., Cys, His)

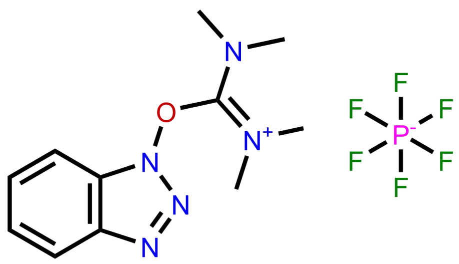
- **Racemization Control**:

Low racemization risk

- **Precautions**:

Irritating to eyes, respiratory tract, and skin; wear eye/face protection.

2.2 HBTU



O-Benzotriazole-N,N,N',N'-tetramethyl-uronium-hexafluorophosphate (HBTU, CAS: 94790-37-1, molecular formula $C_{11}H_{16}N_5OPF_6$, MW: 379.247) is a uronium salt coupling reagent for amide condensation. It is cheaper than HATU but more expensive than the HBOT/EDCI combination. Its addition method is simpler than HBOT/EDCI. The coupling byproducts are HOBT and TMU. Studies show that HBTU exists as a guanidinium cation (not uronium cation) in both crystalline and solution states. As a peptide coupling reagent, its advantages are: extremely low racemization probability, simple reaction conditions, short reaction time, high yield.

- **Reaction Conditions:**

Temperature: 20–25°C

Solvent: DMF (tolerates <1% water)

Base: DIPEA, etc.

- **Applicable Scope:**

Standard SPPS (Fmoc/tBu strategy), solution-phase synthesis (side-chain protection required)

- **Racemization Suppression:**

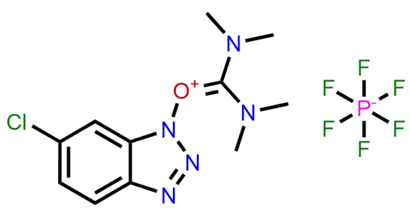
Requires HOBt, etc.

- **Precautions:**

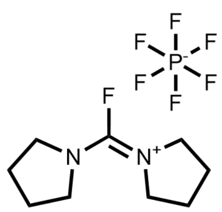
Avoid skin/eye contact – wear protective gear; prevent dust inhalation

2.3 Others

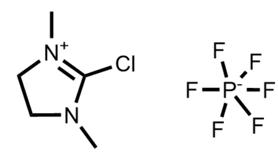
Includes HCTU, BTFFH, CIP, TBTU, TSTU, TNTU, etc. (See figure below)



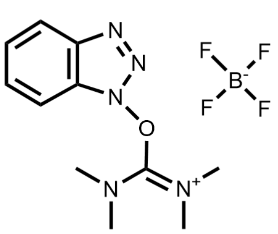
HCTU



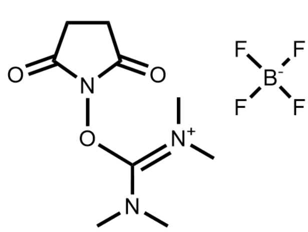
BTFFH



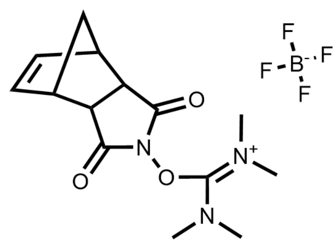
CIP



TBTU



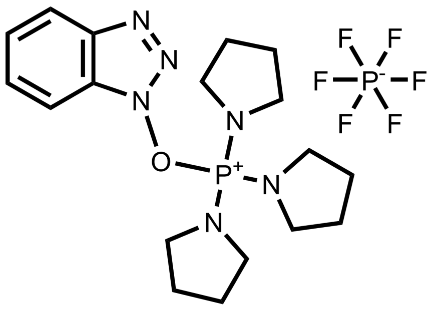
TSTU



TNTU

3. Phosphonium Salt Reagents

3.1 PyBOP



PyBop

Early phosphonium reagents like BOP (tri(dimethylamino)benzotriazol-1-yloxyphosphonium hexafluorophosphate) produced carcinogenic HMPA (hexamethylphosphoramide) byproducts. Consequently, PyBOP has replaced it due to superior reactivity and non-carcinogenic byproducts.

Benzotriazol-1-yl-oxytripyrrolidino-phosphonium Hexafluorophosphate (PyBOP, CAS: 128625-52-5, molecular formula $C_{18}H_{28}F_6N_6OP_2$, MW: 520.4) is a strong coupling reagent with relatively high reactivity among phosphonium salts. Its lower cost compared to PyAOP makes it widely applicable in peptide and amide synthesis. Key advantages include high chemoselectivity (favors N-acylation over O-acylation). The reaction mechanism involves carboxylate anion attack on the reagent under basic conditions to form an acyloxy -phosphonium cation. Benzotriazole oxide anion then attacks this intermediate to generate a benzotriazole ester, which reacts with the amine component to yield the target product.

- **Reaction Conditions:**

Temperature: 0 – 25 ° C (30 ° C for hindered substrates)

Time: 5 – 30 min (2 – 12 hr for cyclic/sterically hindered peptides)

Solvent: Anhydrous DMF/DCM

Base: DIPEA, etc.

- **Applicable Scope:**

Sterically constrained peptides, cyclic peptides (head-to-tail/side-chain cyclization)

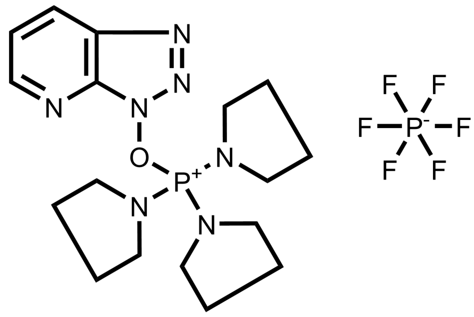
Racemization Suppression:

Add HOAt, etc.

- **Precautions:**

Skin irritant – use protective equipment

3.2 PyAOP



PyAOP

(3-Hydroxy-3H-1,2,3-triazolo[4,5-b]pyridinato-O)tri-1-pyrrolidinylphosphonium hexafluorophosphate (PyAOP, CAS:156311-83-0, molecular formula: $C_{17}H_{27}F_6N_7OP_2$, MW:521.38). It was originally designed as an activator for carboxylic acids but recently found uses in the field of functionalization of intact trimetaphosphate. It is a colorless crystals, soluble in CH_2Cl_2, $CHCl_3$, DMF, DMSO, NMP, THF, CH_3CN, and acetone.

As a reagent-activator for carboxylic acids in peptide synthesis, it doesn't react with primary amines, in contrast to the corresponding uronium/aminium salts which lead to guanidino derivatives. PyAOP is among the most reactive coupling reagents with strong stability, which makes it less effective in automated solid-phase peptide synthesis. The functionalization of trimetaphosphate can be done with primary alcohols, amines, secondary amines, and Wittig reagents to form phosphorus ylide in moderate yield. The reagent can be applied to the triphosphorylation of nucleosides.

- **Reaction Conditions:**

Temperature: 20–25°C

Time: 10–30 min (≤1 min for fast activation)

Solvent: DMF

Base: DIPEA, etc.

- **Applicable Scope:**

N-methyl amino acids, hindered sequences (Aib), pharmaceutical intermediates

Special Handling:

Trp: Argon protection + dark conditions (prevents C2 oxidation)

His: Trt-protected imidazole required

Cys: Disulfide formation risk – avoid unprotected thiols

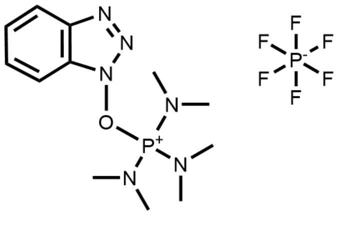
Asp/Glu: Side-chain protection mandatory

- **Racemization Suppression:**

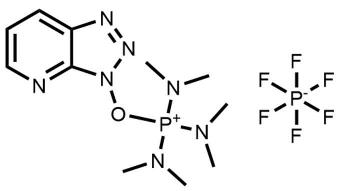
The steric hindrance of tripyrrolidinophosphine leads to self-inhibited racemization.

3.3 Others

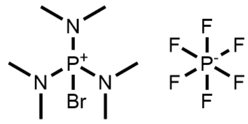
Includes BOP, AOP, PyBroP, FDPP, DEPBT, DPP-Cl, BOP-Cl, etc. (See figure below).



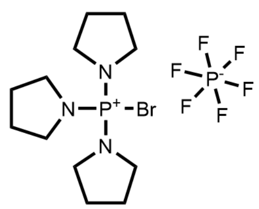
BOP



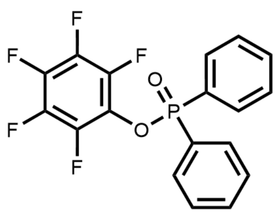
AOP



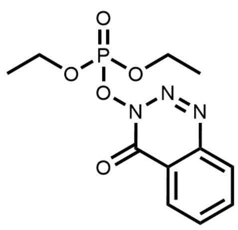
BROP



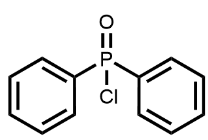
PyBroP



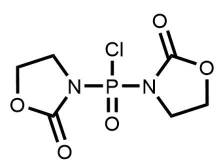
FDPP



DEPBT



DPP-Cl



BOP-Cl

4. Emerging Coupling Reagents

In recent years, advances in novel peptide coupling reagents have empowered chemists to construct amide bonds and design innovative peptide molecules with heightened efficiency, simultaneously driving the field toward greener alternatives. The emergence of new reagents and strategies has provided more environmentally friendly and efficient solutions for amide bond formation. Current green coupling reagents include: TBEC (N-tert-butyl-N'-ethylcarbodiimide), NDTP (5-nitro-4,6-dithiocyanatopyrimidine), etc.

Future development priorities for green coupling reagents focus on:

- High-Efficiency/Low-Toxicity Reagents:

Design rapid-reacting (≤ 1 minute), non-irritating reagents integrated with ynamide-mediated unprotected amino acid synthesis strategies, reducing energy consumption and chemical waste.

- Economic Optimization:

Minimize protecting group usage and byproduct generation through improved activation mechanisms.

- Green Solvent Compatibility:

Promote synergistic applications with solvents like DMM (dimethoxymethane) and carbonate esters to establish low-toxicity, biodegradable peptide synthesis systems.

- Industrial Scalability:

Enhance compatibility with Fmoc strategies and automated production equipment (e.g., fully automated peptide synthesizers), while ensuring stability in green solvents for industrial-scale manufacturing.

These advances have not only significantly enhanced the sustainability of peptide synthesis but also accelerated the widespread clinical adoption of peptide therapeutics. With continuous innovation in green coupling reagents and solvent systems, peptide drug R&D and manufacturing are poised for systematic breakthroughs across three critical dimensions: environmental compatibility, cost-effectiveness, and operational efficiency.