The coupling reaction i.e. the formation of an amide bond between amino acids and/or peptides is the crucial step in peptide synthesis. The reaction consists of two consecutive steps:
1. Activation of the carboxy moiety
2. Acylation of the amino group

During the first step the protected amino acid (or peptide) reacts with a so-called coupling reagent yielding a reactive intermediate. The chemistry behind and the most important coupling reagents will be presented in this brochure.

**Activation during Peptide Synthesis**

The formation of an amide bond between a carboxylic acid moiety and an amino function of two amino acids is the core reaction in peptide synthesis:

The first step of this condensation reaction, the activation of the carboxyl moiety (I. in Fig. 1) is often the critical one. Depending on the type of activating reagent, the intermediate A is a stable compound which could be isolated if desired. During the second step (II. in Fig. 1), A is attacked by a nucleophile such as the α-amino group of a carboxy-protected amino acid. Steps I and II can be performed either consecutively or, with certain types of coupling reagents, as a one-pot reaction.

Over the years, numerous activation methods have been developed, such as the generation of carboxylic halides (chlorides, fluorides), carboxylic azides, symmetrical or mixed anhydrides or the use of carbodiimides (DCC, DIC, EDC·HCl) with or without additives.
If the amino compound contains other functional groups able to take part in the coupling reaction, use of a stable preactivated carboxylic compound A is recommended. Active esters such as the pentafluorophenyl (OPfp) and hydroxysuccinimido (OSu or NHS) esters are reactive amino acid derivatives finding broad application in peptide synthesis. For our comprehensive offer of active esters please see our online shop.

**Coupling Reagents**

Carbodiimides have been used as activators for decades in solid-phase and solution peptide synthesis. They still hold their place, though in recent years two classes of coupling reagents became popular, the phosphonium- and the aminium-(imonium-) type reagents such as BOP, PyBOP, PyBrOP, TBTU, HBTU, HATU, COMU, and TFFH. These compounds achieve high coupling rates accompanied by few undesired side reactions. In contrast to activation by carbodiimides, peptide couplings using the latter compounds require the presence of a base. Diisopropylethylamine (DIPEA) and N-methylmorpholine (NMM) are the most frequently used ones in Fmoc/tBu-based solid-phase synthesis. In cases of a markedly increased risk of racemization, the weaker base sym.-collidine has been recommended for substituting DIPEA or NMM. Racemization is one of the main side reactions, when activating carboxyl groups of amino acids (except for glycine or proline). Electron-withdrawing groups bound to the α-amino moiety (e.g. acyl, peptidyl (i.e. peptide fragments)) increase the...
tendency to racemize considerably. Urethane derivatives, which include standard α-amino protecting groups as Fmoc, Boc, and Z, of amino acids usually retain their optical purity upon activation. The mechanism of racemization (Fig. 2) involves the abstraction of the α-hydrogen from the α-carbon atom of the activated amino acid, either by direct formation of an enolic intermediate (direct α-abstraction, path A) or by formation of a 5-membered oxazolinone ring (path B), which isomeric aromatic configuration is readily formed in the presence of bases.

Many other side reactions have been described so far, depending on the type of side chain functionalities, the combination of protecting groups, the reactivity of the carboxyl group, or the basicity of the amino function. They will be mentioned later as special information for the respective coupling reagent.

Every coupling reagent has its individual advantages and drawbacks. None of them will render good results for all types of amino acid derivatives!

This brochure gives information on a large number of currently available coupling reagents, their scope and limitations. We hope that it can help the chemist to make the appropriate choice.

**Coupling Reagents and Additives**

1. **Carbodiimides**

**DCC** *(Dicyclohexylcarbodiimide)*

This popular condensation reagent has been applied for coupling since 1955 and is still much in use today. DCC is routinely applied in solution as well as in solid-phase peptide synthesis, mostly in combination with additives such as HOBt or HOSu in order to reduce epimerization in the case of peptides or racemization in the case of amino acids. Couplings in solution with amino acid derivatives provided as salts (e.g. hydrochlorides) require one equivalent of a tertiary base (as NMM). Else, the reaction does not require additional base, so that racemization can be kept minimal.

Application of DCC in solid-phase synthesis is limited due to the by-product dicyclohexylurea (DCU) with is formed simultaneously with the coupling reaction (see Fig. 3). This by-product is sparingly soluble in most solvents, and therefore DCC should better be replaced by other carbodiimides e.g. DIC (diisopropylcarbodiimide).
However, in the manufacture of active esters, DCC is still one of the reagents of choice. In solution, the low solubility of DCU turns into an advantage of this activation method. Application of preformed OPfp esters reduces the risk of concomitant racemization during couplings. Fmoc-AA-OPfp-esters can be isolated and purified by crystallization. They are stable but highly reactive building blocks routinely used for couplings in fully automated SPPS.

**Literature:**
- G. W. Anderson and F. M. Callahan, J. Am. Chem. Soc. 80, 2902 (1958)

**DIC**
(Diisopropylcarbodiimide)

DIC (or DIPCDI) is a useful reagent for automated SPPS, because the corresponding urea is soluble in standard solvents such as isopropanol and can be washed out more readily than the one obtained from DCC. If base-free conditions are required as to minimize racemization, the combination of DIC and HOBt (or HOAt, Oxyma Pure) is still one of the best methods e.g. for coupling Fmoc-Cys(Trt)-OH.

**Literature:**

**EDAC · HCl, EDC · HCl, WSC · HCl** (Q-1955)
(N-(3-Dimethylaminopropyl)-N’-ethylcarbodiimide · HCl)

This water-soluble carbodiimide was especially designed for couplings in aqueous solution, even though the stability of the
reagent under these conditions is limited. Normally, EDAC-mediated couplings are performed in polar solvents such as DMF or NMP or even in methylene chloride. EDAC has also been employed in SPPS on highly polar resins compatible with water-containing solvents. Contrary to DCU, the urea formed from EDAC is readily soluble. EDAC is the reagent of choice for the conjugation of peptides, labels, small organic molecules etc. to proteins.

Literature:

In addition to racemization another side reaction with carbodiimides is an O-N-migration of the activated carboxyl function forming an N-acyl urea (see Fig. 3). This stable compound is unable to take part in further couplings reaction. As this side reaction depends on the temperature and coupling behavior of the corresponding amino function, low temperatures are always recommended in carbodiimide-mediated couplings.

Due to the fact, that all carbodiimides are condensing reagents, side reactions of unprotected amino acid-side chains (Asn, Gln), such as conversion of amides to nitriles have been observed. Appropriate side-chain protecting groups will prevent these side reactions.

Other side reactions depending on the nature of additives are not a special problem of carbodiimides and will be described later.
2. Additives

Additives such as HOBt, HOAt or Oxyma pure® are strongly recommended in all cases of amide bond formations with carbodiimides, in order to enhance the reactivity and also to reduce formation of epimers as well as N-acylureas.

HOBt
(1-Hydroxybenzotriazole)

N-Hydroxybenzotriazole, developed by W. König and R. Geiger in 1970 was the most popular additive during the last decades. Today, HOBt is still one of the most effective suppressors of racemization in carbodiimide-mediated reactions. The most important drawback of the additive lies in its explosive character, especially in water-free form. Therefore, its availability is more restricted today and it should be substituted by more stable additives in the future. The mechanism of activation by HOBt used in combination with DCC is shown in Fig. 4.

Literature:

HOBt-6-sulfonamidomethyl resin · HCl
(200-400 mesh) (D-2435)
(1-Hydroxybenzotriazole-6-sulfonamido-
methyl resin · HCl)

“Polymeric HOBt”, a highly efficient polymeric auxiliary for the synthesis of amides. Simple filtration allows the separation of the product from the polymer.

Literature:

HOOBt (HODhbt)
(Hydroxy-3,4-dihydro-4-oxo-1,2,3-benzotriazine)

ODhbt esters, generated during coupleings with a carbodiimide and HOOBt or DEPBT-mediated couplings, are more reactive than HOBt esters. The release of the benzotriazine can be monitored spectrophotometrically.

Literature:

HOSu (Q-1800)
(N-Hydroxysuccinimide)

In contrast to HOBt and HODhbt, HOSu is completely shelf-stable. It is also a well-known additive in carbodiimide-mediated reactions since many years. On the other hand HOSu forms stable and isolable active esters, which are applicable in organic as well as in aqueous solution. A drawback of HOSu is due to its hydroxamic acid structure susceptible to the Lossen-rearrangement. This side reaction can be observed under condensation conditions and leads to introduction of an additional β-alanine.

Literature:
**HOAt**

(1-Hydroxy-7-aza-1H-benzotriazole)

More than HOBt, this additive accelerates coupling reactions and suppresses racemization. However, the explosive properties of this compound restrict its applications and availability.

**Literature:**


**Oxyma Pure® (Q-2750)**

(Ethyl 2-cyano-2-(hydroximino)acetate)

A trademark of Luxembourg Bio Technologies Ltd, Rechovot, Israel

This more recently developed additive is a non-explosive alternative to HOBt or HOAt, and allows high coupling rates at low racemization when applied in combination with carbodiimides.

In practice, Oxyma Pure can be used in an identical manner as HOBt in DMF on automated synthesizers.

**Literature:**


**3. Phosphonium Reagents**

**BOP (Q-1980)**

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

BOP was the first of a broad range of phosphonium type coupling reagents. It was introduced by Castro et al. already in 1975. BOP provides excellent coupling behavior and good solubility in most of the common solvents, in solid-phase as well as in solution. It converts carboxyl groups into -OBt esters and has no guanylation-activity to amino functions as aminium-compounds
like TBTU. It is a useful reagent for lactonization, selective esterification or amidation of α-amino acids without racemization. However, its severe drawback is the toxicity problem due to the carcinogenic HMPA (hexamethylphosphoramide) formed as by-product during the reaction.

**Literature:**

**PyBOP® (Q-2715)**
(Benzotriazol-1-yl oxy-tripyrrolidino-phosphonium hexafluorophosphate)

PyBOP® is a non-toxic version of BOP. It has the same effective coupling properties in solid phase as BOP. PyBOP has been used as well for obtaining peptide thioesters.

**Literature:**

**PyBrOP®**
(Bromo-tripyrrolidino-phosphonium hexafluorophosphate)

A trademark of Merck KGaA, Darmstadt, Germany
This reagent was developed by J. Coste, to overcome the lack of PyBOP®, as well as other HOBt-containing coupling reagents, in incomplete couplings to N-methyl-amino-acids. Further good results were reported in coupling of Aib-derivatives. Additionally, due to its high reactivity, PyBrOP® is not a standard coupling reagent for all amino acids. The formation of oxazolones during prolonged couplings accompanied by higher racemization limits the use of the coupling reagent.

**Literature:**

**PyAOP®**
(7-Aza-benzotriazol-1-yl oxy-tripyrrolidino-phosphonium hexafluorophosphate)

The HOAt-analog to PyBOP®, developed by L. Carpino gave also remarkable faster coupling rates as PyBOP®, due to the enhanced electron withdrawing effect of the corresponding formed -OAt active esters during coupling reaction.
Contrary to HATU PyAOP cannot react with amino groups yielding guanidines.

**Literature:**

**PyOxim (Q-2760)**
(Ethyl cyano(hydroxyimino)acetato-O2)-tri-(1-pyrrolidinyl)-phosphonium hexafluorophosphate)

This recently developed coupling reagent contains Oxyma pure as part of the molecule instead of the explosive compounds HOBt or HOAt. This improvement of safety in handling is accompanied with an accelerated reactivity in couplings as well as a minimized allergenic potential, making PyOxim to one of the best coupling reagents in solid-phase reactions. The only drawback is the formation of tris-pyrrolidinophosphamide as side product, which can cause problems in separation when the reagent is applied in solution.

**Literature:**

**DEPBT (Q-2565)**
(3-(Diethoxy-phosphoryloxy)-1,2,3-benzo[d] triazin-4(3H)-one)

Contrary to the other described phosphonium reagents, DEPBT is a mixed anhydride of H00Bt and diethylphosphoric acid. It mediates amide bond formations with a remarkable resistance to racemization. Therefore it is the reagent of choice for coupling the racemization prone Fmoc-His(Trt)-OH.

**Literature:**

Phosphonium-based coupling reagents do not affect amino groups and are well suited to be used as cyclization reagents, when applied in excess. Two classes of building blocks should be disclaimed from coupling procedures with phosphonium reagents:

a. Phosphorylated amino acids (Fmoc-Ser(PO,OH,OBzl)-OH,…) can undergo undesired couplings between reagent and the unprotected phosphoryl-side chain.

b. Compounds containing nucleotides (e.g. PNA-building blocks) with oxo-functions like guanine, etc. are prone to be attacked under structural rearrangement.

**Literature:**

4. Aminium/Uronium-Imonium Reagents

**TBTU (BF4−) (Q-1665)/ HBTU (PF6−)**
(2-(1H-Benzotriazol-1-yl)-N,N,N’N’-tetramethylammonium tetrafluoroborate/ hexafluorophosphate)
These two compounds, only differing in their counterions (BF₄⁻, PF₆⁻) have nearly identical chemical properties and belong to the most popular coupling reagents. Their application is widespread in solid-phase reactions as well as in solution, because all resulting by-products are soluble in water as well as in standard organic solvents (see Fig. 5). For couplings of phosphorylated amino acids TBTU and HBTU are the reagents of choice.

**Literature:**

A well-known limitation of aminium/uronium-derivatives is a possible reaction with free amino groups yielding guanidines, when the coupling reagent is applied in excess (Fig. 6). This side reaction is normally inhibited by application of a slight excess of carboxyl compound in relation to coupling reagent and by a short period of preactivation before adding to the amino compound.

**HCTU**
(2-(6-Chloro-1H-benzotriazol-1-yl)-N,N,N',N'-tetramethylaminium hexafluorophosphate)

The replacement of HOBT by 6-Chloro-HOBT as part of the molecule, leads to higher reaction rates and improved results in the synthesis of difficult peptides.

**Literature:**
O. Marder, Y. Shvo, F. Albericio, Chimica Oggi 20, 37 (2002)
O. Marder and F. Albericio, Chimica Oggi 21, 6 (2003)
**Coupling Reagents**

**HDMC** (Q-2765)
(N-[(5-Chloro-1H-benzotriazol-1-yl)-dimethylamino-morpholino]-uronium hexafluorophosphate N-oxide)

When modifying the uronium part of HCTU by introduction of a morpholine moiety, a further increase of reactivity can be gained. Even coupling rates of HATU could be exceeded in some cases by HDMC.

*Literature:*

**TATU** ($\text{BF}_4^-$) (Q-2150)/ **HATU** ($\text{PF}_6^-$) (Q-2780)
(2-(7-Aza-1H-benzotriazol-1-yl)-N,N,N',N'-tetramethylammonium tetrafluoroborate/hexafluorophosphate)

The HOAt analogs to HBTU/TBTU are highly efficient coupling reagents for solid- and solution-phase reactions. Contrary to HOBt-based reagents they have been used successfully in couplings of N-methyl-amino acids.

*Literature:*

**COMU** (Q-2735)
(1-[1-(Cyano-2-ethoxy-2-oxoethylidene-aminoxy)-dimethylamino-morpholino]-uronium hexafluorophosphate)

COMU is a novel coupling reagent with coupling efficiencies comparable to HATU. The incorporation of Oxyma Pure as part of the molecule in place of the explosive compounds HOBt or HOAt results in safer handling in combination with better solubility and a reduced allergic potential than HBTU/TBTU or HATU. COMU is especially suited for microwave-accelerated SPPS. Recently it has been demonstrated that COMU can be used to prepare esters of all types of alcohols at room temperature under mild conditions in the presence of organic bases.
**Literature:**

**TOTT (Q-2600)**
(2-(1-Oxy-pyridin-2-yl)-1,1,3,3-tetramethyl-isothiouronium tetrafluoroborate)

TOTT is a thiuronium salt of 2-mercaptopyridone-1-oxide which showed good results in couplings of sterically hindered or methylated amino acids, comparable with HATU. Racemization levels of those couplings are reported to be lower as with other reagents.

**Literature:**

**TFFH (Q-2280)**
(Tetramethylfluoroformamidinium hexafluorophosphate)

A highly efficient coupling reagent which generates amino acid fluorides in situ. Amino acid fluorides are especially suited for the coupling of sterically hindered α,α-disubstituted amino acids such as Aib.

**Literature:**
A. El-Faham and S.N. Khattab, Synlett 886 (2009)

5. Miscellaneous Coupling Reagents

**EEDQ (Q-1735)**
(N-Ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline)

EEDQ is an “old” reagent (developed in 1967), which still is of interest due to its re-
Coupling Reagents

Markable properties. It converts the amino acid derivative into an anhydride EEDQ does not require the presence of a tertiary base nor does it affect the amino function or other reactive groups such as hydroxyls (Fig. 7). Albeit the formation of the anhydride is slow, it is consumed very rapidly by the amino-component which minimizes racemization during product formation. As an example, EEDQ was the only reagent amongst the tested ones which mediated the coupling 3,5-dinitrobenzoyl-Leu-OH to 3-aminopropyl-silica with negligible level of concomitant racemization.

Literature:
B. Belleau and G. Malek, J. Am. Chem. Soc. 90, 1651 (1968)

T3P
(2-Propanephosphonic acid anhydride)

A trademark of Euticals
T3P (or PPA) is a cyclic anhydride of propylphosphonic acid, which is normally used in combination with tertiary amines for solution-phase and cyclization reactions. PPA gives superior results especially for sterically hindered peptides.

Literature:

DMTMM and related compounds
(4-(4,6-Dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium salts)

Triazines - a group of coupling reagents, developed by Kaminski et al. - are condensation products of substituted cyanuric
chloride with tertiary amines as NMM or DABCO, able to mediate peptide couplings in water or alcoholic solutions. These reagents proved to be particularly efficient allowing high yields and low racemization levels. For the coupling mechanism of DMTMM see Fig. 8.

Literature:

BTC
(bis-Trichloromethylcarbonate or “Triphosgene”)

The stable trimeric form of phosgene is a well-known reagent for generating acid chlorides from carboxylic acids. Its applicability for peptide couplings was evaluated by C. Gilon et al. for solution- and solid-phase couplings. They used collidine as base and THF or DCM as solvents. Other solvents such as DMF or NMP have to be strictly avoided, as they can react with BTC. Care must be taken when handling the reagent, because BTC and even more so phosgene which is formed as intermediate are highly toxic compounds. Beyond these restrictions, BTC is one of the most efficient activation reagents and recommended especially for difficult couplings and less reactive amines such as anilines.

Literature:

CDI
(1,1’-Carbonyldimidazole)

CDI, as the related compounds disuccinimidyld carbonate or 4-nitrophenyl-chlorofor-mate, is a phosgene analog, a derivative of carbonic acid. CDI is not routinely used for peptide couplings. Its field of application lies in the synthesis of ureas and urethanes from amines, alcoholic compounds or resins and linkers. Nevertheless CDI has been successfully used in peptide couplings. The protocol for couplings has to include a preactivation step during which the reactive acid imidazolidine is formed, which is added to the amino component.

Literature:

Conclusion

TBTU, HBTU and PyBOP are well suited reagents for most standard coupling reactions. They all contain the potentially explosive HOBT as part of the molecule. The same problem is encountered with the more reactive HOAt-based coupling reagents. Due to safety considerations, all of them will have to be replaced in the future by OxyMa Pure-based reagents such as COMU or PyOxim. More specialized reagents as HATU, HDMC, TOTT or DEPBT can be required to succeed in incorporating amino acid derivatives prone to side reactions. However, “old” reagents as EEDQ still can be useful in cases where other reagents rendered poor results. Bachem offers a large selection of coupling reagents to meet all requirements of the synthetic chemist.
REFERENCES

For more information as well as many valuable hints regarding properties and application of coupling reagents the following review articles are recommended:

**F. Albericio and L.A. Carpino**

**F. Albericio, R. Chinchilla, D.J. Dodsworth, C. Nájera**

**S.-Y. Han and Y.-A. Kim**

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**C.A.G.N. Montalbetti and V. Falque**

**E. Valeur and M. Bradley**

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**C. Roche, M. Pucheault, M. Vaultier, A. Commerçon**

**A. El-Faham and F. Albericio**

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**T.I. Al-Warhi, H.M.A. Al-Hazimi, A. El-Faham**

**R. Subirós-Funosas, S.N. Khattab, L. Nieto-Rodríguez, A. El-Faham, F. Albericio**

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COUPLING REAGENTS

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Q-1665

**TFFH**
(Tetramethylfluoroformamidinium hexafluorophosphate)
Q-2280

**TOTT**
(2-(1-Oxy-pyridin-2-yl)-1,1,3,3-tetramethylisothiouronium tetrafluoroborate)
Q-2600

ADDITIVES

**HOSu**
(N-Hydroxysuccinimide)
Q-1800

**Oxyma Pure**
(Cyano-hydroxyimino-acetic ethyl ester)
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