Peptide Reagents

The creation of a peptide linkage between two amino acid segments is one of the most important reactions in organic and bioorganic chemistry. Many different strategies have been devised for selective amide bond formation from a carboxylic acid and an amino group, usually involving protection, activation, coupling and deprotection steps. The reagents listed in this section have been used in one or other of these steps in the synthesis of peptide molecules. An enormous body of literature exists on the subject of peptide synthesis, of which a selection of books and review articles is listed.[1-12]

Protecting group reagents

To ensure specific coupling between the required carboxyl and amino groups, a range of protecting groups have been developed which can be selectively introduced and removed. References to methods of protection and deprotection can be found under specific items in the main Catalogue. Further information on the use of protecting groups in peptide synthesis can be found in the general references on peptide synthesis and in other specialist sources.[13-15]

CARBOXYL PROTECTION

Although a wide variety of esters and other groups have been used for the protection of carboxylic acids, only a limited number of these find significant use in peptide synthesis.

tert-Butyl ester

L08855 tert-Butyl acetate
L00431 N,N-Dimethylformamide di-tert-butyl acetal
B22039 tert-Butyl 2,2,2-trichloroacetimidate
L12338 tert-Butanol

1-Adamantyl ester

A10209 1-Adamantanol

Dicyclopropylmethyl ester

L01816 Dicyclopropylmethanol

Benzylic esters

A15188 9-Anthracenemethanol
L03292 Benzyl alcohol
A13535 Benzyl bromide
A12481 Benzyl chloride
A13579 9-(Chloromethyl)anthracene
A12859 4-(Chloromethyl)pyridine hydrochloride
A15212 9-Fluorenylmethanol
A15559 4-Methoxybenzyl alcohol
A15742 4-Nitrobenzyl alcohol
A13127 2-Nitrobenzyl bromide
A15236 4-Nitrobenzyl bromide
A15749 4-Nitrobenzyl chloride
L00555 2,3,4,5,6-Pentamethylbenzyl chloride
A14698 Pyridine-4-methanol
L00423 2,4,6-Trimethylbenzyl chloride

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Benzhydryl-type ester precursors

A12266 Benzophenone hydrazone
B21706 9-Fluorenone hydrazone

Phenacyl esters

A15576 2-Bromoacetophenone
A13415 2-Bromo-4'-methoxyacetophenone

Allyl ester

A15026 Allyl alcohol
A11766 Allyl bromide

2-Substituted ethyl esters

A10275 2-Bromoethanol
B23579 2-(2-Hydroxyethyl)pyridine
L05442 2-(Methylthio)ethanol
L19365 2-(p-Toluenesulfonyl)ethanol
L08163 2,2,2-Trichloroethanol
B20970 2-(Trimethylsilyl)ethanol

Miscellaneous carboxyl protecting reagents

A13005 Benzyl carbazate
A12383 tert-Butyl carbazate
A15238 2-Chloroacetamide
A12018 N-(Chloromethyl)phthalimide
B21292 N-(Hydroxymethyl)phthalimide

AMINO PROTECTION

The most popular protecting groups for the amino function are carbamates, particularly Benzyloxycarbonyl [Cbz, Z], Boc and Fmoc groups, but many other groups including trifluoroacetyl and trityl can be used.

Benzyl carbamate [Cbz, Z]

A15682 Benzyl chloroformate
A12153 N-(Benzyloxycarbonyloxy)succinimide

Substituted benzyl carbamates

B25632 4-Nitrobenzyl chloroformate

tert-Butyl carbamate [Boc]

A14708 Di-tert-butyl dicarbonate
L00506 N-Boc-imidazole 2-phenylacetonitrile]
A12383 tert-Butyl carbazate
A18906 Boc-ON [2-(tert-Butoxycarbonyloximino)
B22435 S-Boc-2-mercapto-4,6-dimethylpyrimidine

Benzo[b]thiophenesulfoylmethyl carbamate [Bsmoc]

L19559 1,1-Dioxobenzo[b]thiophen-2-ylmethyl chloroformate
L19733 1,1-Dioxobenzo[b]thiophen-2-ylmethyl N-succimidyl carbonate

2,2,2-Trichloroethyl carbamate [Troc]

L06875 2,2,2-Trichloroethyl chloroformate

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9-Fluorenenylmethyl carbamate [Fmoc]

A11683 9-Fluorenylmethyl chloroformate

A13143 N-(9-Fluorenylmethoxycarbonyloxy)-succinimide

Allyl carbamate [Alloc]

B22332 Allyl chloroformate

Trifluoroacetyl group

A13614 Trifluoroacetic anhydride

L00135 Trifluoroacetylimidazole

Trityl groups (Trt, Mmt)

A12864 Bromotriphenylmethane
A11799 Chlorotriphenylmethane

A10545 4-Methoxytrityl chloride

Miscellaneous amino protecting groups

A14822 N-(Ethoxycarbonyl)phthalimide
L06432 Chlorocarbonylsulfenyl chloride
L00770 2-Chloro-3,5-dinitropyridine

B23627 2-Nitrobenzenesulfenyl chloride
A15857 3-Nitrophthalic anhydride

SIDE CHAIN PROTECTION

The presence of reactive side chains in certain amino acids can interfere with peptide synthesis. Some examples are given here of reagents used to mask this reactivity:

Hydroxyl protection

Of the vast range of groups available for the blocking of hydroxyl groups, only a few find regular use in peptide synthesis for serine, threonine and tyrosine derivatives. Benzyl ethers (from benzyl halides or alcohol) and tert-butyl ethers are widely used. For silyl protection, see Appendix 4. Among the most useful OH protecting reagents are:

L13471 N-(2-Bromobenzyloxycarbonyloxy)-succinimide
A12387 Benzyl 2,2,2-trichloroacetimidate
A10370 3-Bromocyclohexene

L13576 3-Bromopentane
B22039 tert-Butyl 2,2,2-trichloroacetimidate
A12859 4-(Chloromethyl)pyridine hydrochloride
L01050 2-Methoxyethoxymethyl chloride

Thiol protection

A variety of methods have been employed to mask the thiol group of cysteine, including benzyl, substituted benzyl, benzhydryl, trityl, Cbz and Boc groups.[16] Examples of reagents for SH protection are given:

A12884 Benzhydrol
A13820 N-(Hydroxymethyl)acetamide
L14367 trans-β-Nitrostyrene

A10366 Triphenylmethanol
L13316 4-Vinylpyridine

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Carboxyl protection

The side chain carboxyl groups of aspartyl and glutamyl residues can be blocked by means of one of the reagents listed above for carboxyl protection, and also:

A10697 2-Adamantanol

A10381 Pyridine-3-methanol

Guanidino protection

The guanidino group of arginine has been protected by N-nitration, or by Cbz or Boc derivatives, amongst others. Arylsulfonyl protection has been found to be particularly useful:

A11775 Mesitylenesulfonyl chloride

L11829 4-Methoxy-2,3,6-trimethylbenzenesulfonyl chloride

L19561 2,2,4,6,7-Pentamethyldihydro-benzo[b]furan-5-sulfonyl chloride

Imidazole protection

The imidazole ring in histidine can interfere with acylation reactions and can also promote racemization. It has been blocked by a variety of methods including formation of the Boc or Troc derivatives (reagents listed under Amino Protection) and the following:

A11871 2,4-Dinitro-1-fluorobenzene

A13788 4-Methoxybenzenesulfonyl chloride

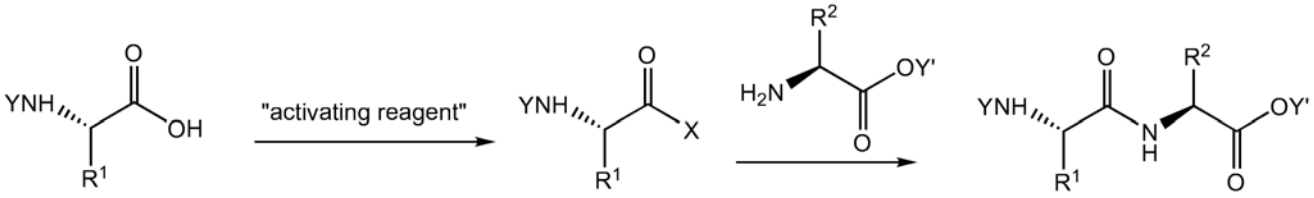
A13127 2-Nitrobenzyl bromide

A14547 p-Toluenesulfonyl chloride

Activation and coupling methods

Formation of the peptide link under sufficiently mild conditions normally requires activation of the carboxylic acid function by conversion to a more electrophilic species, such as an acyl halide, azide, anhydride, mixed anhydride or active ester, which then undergoes coupling *in situ*, or as a separate step, with the amino function of the second component, as shown in Scheme 1.

Scheme 1



Y and Y' are protecting groups; X is a leaving group

Acid chlorides have limited value in peptide coupling because of the danger of hydrolysis, racemization, cleavage of protecting groups and other side reactions.[12] These difficulties can generally be avoided by the use of acid fluorides as active intermediates.

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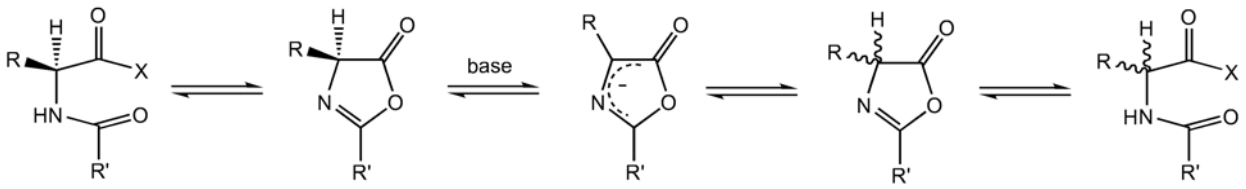
Mixed anhydrides of various kinds, formed by reaction of a carboxylic acid with chloroformates, acid chlorides, etc., in the presence of a tertiary amine base, have a long history as peptide intermediates. A variety of phosphorus-based reagents have been employed to form mixed anhydrides and some have shown promising results, often with simplified procedures.

However, for more than four decades the most widely-used reagents have been carbodiimides, especially N,N-dicyclohexylcarbodiimide [DCC] and water-soluble carbodiimide[17] [EDCI, "WSC"]. These will couple protected amino acids directly, but better results are often obtained with various additives or activating agents, most of which can form an "active ester", containing a good leaving group, with the carboxyl function. Frequently the coupling is performed as two separate steps: carbodiimide promoted formation (and possible isolation) of the active ester, followed by reaction with the free amino species.

The main problem is often partial loss of chirality caused by side reactions, the most important of which is considered to be azlactone (oxazolinone) formation by intramolecular cyclization of an N-acylated activated acid derivative with proton abstraction at the chiral center (Scheme 2).

Scheme 2



The suppression of racemization has been a major goal of much of the effort in development of coupling methods, and many techniques have been introduced, of which the use of active esters plays a prominent role. Newer coupling methods include the use of pyridinium salts, phosphonium salts and a variety of uronium salt reagents, some of which have been used to form active esters, giving superior results in specific coupling applications. Several other methods of peptide coupling are sometimes used, including the oldest of all, the azide method[11] due to Curtius,[18] of which the use of diphenylphosphonic azide[19] is a variation.

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ACTIVATING AND COUPLING REAGENTS

Further information and literature references on the use of most the following reagents can be found in the text entries in the main section of the Catalogue.

Acyl halides

L18013 Bis(tetramethylene)fluoroformamidinium hexafluorophosphate [BTFFH]
A15666 Cyanuric fluoride
A11992 Diethylaminosulfur trifluoride
A18012 Oxalyl chloride

Carbonic and carboylic mixed anhydrides

A14688 N,N'-Carbonyldiimidazole
A13724 2-Ethoxy-1-ethoxycarbonyl-1,2-dihydro-quinoline [EEDQ]
A14692 Isobutyl chloroformate
L14159 2,4,6-Trichlorobenzoyl chloride
A15051 Trimethylacetyl chloride

Phosphorus mixed anhydrides

L08775 Bis(2-oxo-3-oxazolidinyl)phosphinic chloride [BOP-Cl]
L09919 Diethyl chlorophosphite
A11721 Diphenylphosphinic chloride
A13546 Diphenyl phosphorochloridate
A12724 Ethylene chlorophosphite
A14530 Lawesson's Reagent
L19271 1-Propylphosphonic acid cyclic anhydride, [®T3P], 50+% soln. in DMF
L11911 50+% soln. in ethyl acetate

Sulfonic mixed anhydride

L12147 3,5-Dichloro-2-hydroxybenzenesulfonyl chloride

Carbodiimides

L19463 N-Cyclohexylcarbodiimide on Merrifield resin
L00822 1-Cyclohexyl-3-(2-morpholinoethyl)-carbodiiimide methyl-p-toluenesulfonate
A10973 N,N'-Dicyclohexylcarbodiimide [DCC]
A19292 N,N'-Diisopropylcarbodiimide
B25057 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide
A10807 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride [EDCI]
A10962 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide methiodide
L19462 N-Isopropylcarbodiimide on Merrifield resin

Active ester reagents

A10802 Acetone oxime
L00290 Bis(4-nitrophenyl) carbonate
B24153 2,5-Diphenyl-4-hydroxy-3-oxo-2,3-dihydrothiophene 1,1-dioxide [HOTDO]
L13513 4,6-Diphenylthieno[3,4-d]-1,3-dioxol-2-one 5,5-dioxide [TDO activated carbonate]
A12892 1-Hydroxybenzotriazole hydrate [HOBt]
A13205 endo-N-Hydroxy-5-norbornene-2,3-dicarboximide [HONB]
A13862 N-Hydroxyphthalimide
A14522 2-Hydroxypyridine
A10312 N-Hydroxysuccinimide
A15312 3-Nitrophenol
A14376 4-Nitrophenol
L00359 4-Nitrophenyl trifluoroacetate
A15574 Pentafluorophenol [PFP]
B25671 2,4,5-Trichlorophenol
A10788 2,2,2-Trifluoroethanol

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Pyridinium salts

A12820 2-Chloro-1-methylpyridinium iodide

L11088 2-Fluoro-1-methylpyridinium p-toluenesulfonate

Phosphonium salts

A16140 Benzotriazol-1-yloxytris-(dimethylamino) phosphonium hexafluorophosphate [BOP]

B25251 Benzotriazol-1-yloxytris(pyrrolidino) phosphonium hexafluorophosphate [PyBOP]

L19384 Bromotri(pyrrolidino)phosphonium hexafluorophosphate [PyBroP]

Uronium salts

B23597 O-(1H-Benzotriazol-1-yl)-N,N,N',N'-tetra-methyluronium hexafluorophosphate [HBTU]

L13470 O-(1H-Benzotriazol-1-yl)-N,N,N',N'-tetra-methyluronium tetrafluoroborate [TBTU]

L19494 O-(1,2-Dihydro-2-oxo-1-pyridyl)-N,N,N'N'-tetra-methyluronium tetrafluoroborate [TPTU]

L18496 O-[(Ethoxycarbonyl)cyanomethylene-amino]-N,N,N'N'- tetramethyluronium tetrafluoroborate [TOTU]

L13538 2-Succinimido-1,1,3,3,-tetramethyluronium tetrafluoroborate [TSTU]

Miscellaneous coupling reagents

L14107 Diethyl cyanophosphonate

A12124 Diphenylphosphonic azide

A11118 2,2'-Dipyridyl disulfide

A15087 Ethyl diphenylphosphinite

L14159 2,4,6-Trichlorobenzoyl chloride

BASES

Most coupling reactions require the presence of a tertiary amine base such as triethyl-amine. Milder bases, especially 4-methylmorpholine, are less likely to promote racemization and other side reactions. Non-nucleophilic bases such as N-ethyldiisopropyl-amine (Hünig's Base) are also widely used. The 4-dialkylaminopyridines are frequently used in substoichiometric amounts, in combination with another amine, as hyper-nucleophilic catalysts in mixed anhydride or carbodiimide coupling reactions. Tertiary amine bases are often also required in the introduction of protecting groups.

Tertiary amine bases

A11058 2,4,6-Collidine

L14143 2,6-Di-tert-butyl-4-methylpyridine

L05265 1-Diethylamino-2-propanol

A11081 N-Ethyldiisopropylamine

A11905 4-Ethylmorpholine

A16294 1-Ethylpiperidine

A10478 2,6-Lutidine

A12158 4-Methylmorpholine

L03398 1-Methylpiperidine

L19372 Morpholine, polymer supported [Methylmorpholine on polystyrene]

A12314 1,1,3,3-Tetramethylguanidine

B23797 Tribenzylamine

A12646 Triethylamine

Acylation catalysts

A13016 4-Dimethylaminopyridine [DMAP]

B23989 4-(1-Pyrrolidino)pyridine

A12575 1-Methylimidazole

A11597 1,2,4-Triazole

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MISCELLANEOUS REAGENTS AND SOLVENTS

The Alfa Aesar product range includes many other items useful in peptide chemistry. This section lists a limited number of examples of materials not covered by earlier sections of this Appendix. The Catalogue also contains a comprehensive listing of natural and unnatural amino acids and their protected derivatives.

Deprotecting reagents

The Catalog text entries for the protecting reagents listed in this Appendix give some details of methods of cleavage. Some of the most frequently-used reagents are listed below. For further information, see the general references.[1-15]

L11577 4-(Aminomethyl)piperidine
A15275 Boron trifluoride diethyl ether complex
A14740 Dichloroacetic acid
A14005 Hydrazine monohydrate
A14475 Hydrobromic acid, 45% in acetic acid
L17117 Hydrogen fluoride pyridine complex
A10355 Morpholine
A13565 Methanesulfonic acid
A12623 Palladium, 5% on carbon
A12012 Palladium, 10% on carbon
42578 Palladium hydroxide on carbon [Pearlman's Catalyst]
A12442 Piperidine
L13303 Tetra-n-butylammonium fluoride trihydrate
L06374 Trifluoroacetic acid, 99%
A12198 Trifluoroacetic acid, 99%
A14365 Trifluoroacetic acid, biochem. grade, 9.5+%
A10173 Trifluoromethanesulfonic acid
B21789 Tris(2-aminoethyl)amine
L19373 Tris(2-aminoethyl)amine, polymer-supported

Cation scavengers

Cleavage of protecting groups under acidic conditions often liberates cations (for example tert-butyl, benzyl, or trityl ions) which can undergo unwanted side reactions with the peptide molecule. The following compounds are among the most useful cation scavengers for these reactive species:

A12997 Anisole
L12865 Ethanedithiol
L04163 Pentamethylbenzene
A14846 Thioanisole
A10320 Triethylsilane
L09585 Triisopropylsilane

Solvents

A wide variety of solvents can be used in peptide synthesis. Those listed are among the most useful. Lack of solubility often dictates the use of relatively polar, including dipolar aprotic, solvents. Solvent mixtures can also be used:

A19862 Acetonitrile
L13089 Dichloromethane
A10924 N,N-Dimethylacetamide
A13547 N,N-Dimethylformamide
A13280 Dimethyl sulfoxide
A12747 1,1,1,3,3,3-Hexafluoro-2-propanol
A12260 1-Methyl-2-pyrrolidinone
L13304 Tetrahydrofuran
A10788 Trifluoroethanol

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Miscellaneous reagents and materials

L19593 N-Acryloylsarcosine methyl ester resin [Sheppard acrylamide resin]
L19600 BT-Core resin
L19602 2-Chlorotrityl alcohol on polystyrene
12457 Copper(II) chloride
A10138 1,4-Dithioerythritol
A15797 1,4-Dithio-DL-threitol
B22183 4-(Hydroxymethyl)benzoic acid
L15721 4-(Hydroxymethyl)phenoxyacetic acid
L17027 Merrifield Resin, 1% crosslinked
A16087 Merrifield Resin, 2% crosslinked
L17028 Wang Resin, 1% crosslinked
L19369 Wang Resin, 2% crosslinked

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