Amino Acid-Protecting Groups

Albert Isidro-Llobet,[†] Mercedes Álvarez,[*,†,‡,§] and Fernando Albericio[*,†,‡,||]

Institute for Research in Biomedicine, Barcelona Science Park, Baldiri Reixac 10, 08028 Barcelona, Spain; CIBER-BBN, Networking Centre on Bioengineering, Biomaterials and Nanomedicine, Barcelona Science Park, Baldiri Reixac 10, 08028 Barcelona, Spain; Laboratory of Organic Chemistry, Faculty of Pharmacy, University of Barcelona, 08028 Barcelona, Spain; and Department of Organic Chemistry, University of Barcelona, Martı´ i Franqués 1, 08028 Barcelona, Spain

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* To whom correspondence should be addressed. Fax: 34 93 403 71 26. E-mail: mercedes.alvarez@irbbarcelona.org; albericio@irbbarcelona.org;
† Institute for Research in Biomedicine.
‡ CIBER-BBN.
§ Laboratory of Organic Chemistry, Faculty of Pharmacy, University of Barcelona.
|| Department of Organic Chemistry, University of Barcelona.

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1. Introduction

Synthetic organic chemistry is based on the concourse of reagents and catalysts to achieve the clean formation of new bonds, and appropriate protecting groups are required to prevent the formation of undesired bonds and side reactions.[1,2] Thus, a promising synthetic strategy can be jeopardized if the corresponding protecting groups are not properly chosen.

Emil Fischer was possibly the first to recognize the need to temporally mask a functional group to allow regioselective bond formation in the synthesis of carbohydrates.[3] However, the first "modern" protecting group was the benzylozycarbonyl (Z) developed by Bergmann and Zervas.[4] Z fits with the main characteristics associated with a protecting group: (i) it is easily introduced into the functional group; (ii) it is stable to a broad range of reaction conditions; and (iii) it is safely removed at the end of the synthetic process or when the functional group requires manipulation. Another cornerstone in this field was when Barany et al.[5,6] described the concept of *orthogonality*, in the sense that the two or more protecting groups belong to independent classes and are removed by distinct mechanisms. The groups can be removed,therefore,in any order and in the presence of the rest.

Professor Fernando Albericio was born in Barcelona, Spain, in 1953. He received his Ph.D. in Chemistry at the University of Barcelona, in 1981. Following postdoctoral work at Tufts University (Boston), at the Université d'Aix-Marseille (France), and at the University of Minnesota (1981−1984), he returned to Barcelona as Associate Professor. During the 1992− 1994 period, he was Director of Peptide Research with Milligen/Biosearch in Boston. He rejoined the University of Barcelona, where he was promoted to professor in 1995. Nowadays, he is holding various appointments: General Director of the Barcelona Science Park, Professor at the University of Barcelona, and Group Leader at the Institute for Research in Biomedicine. Professor Albericio's major research interests cover practically all aspects of peptide synthesis and combinatorial chemistry methodologies, as well as synthesis of peptides and small molecules with therapeutic activities. He has published over 500 papers, several review articles, and 22 patents, and he is coauthor of 3 books. He is editor of several scientific journals and acts in the editorial board of several others. In addition, professor Albericio is deeply involved in the development of the third mission of the University, the transference of knowledge and technology to society. He has founded several biotech companies and is acting in the board of directors of several foundations and companies. Furthermore, he is consultant for several companies in the chemical and pharmaceutical areas.

Albert Isidro-Llobet was born in El Prat de Llobregat (Spain) in 1981. He studied Chemistry at the University of Barcelona (Spain) and obtained his Ph.D. in 2008 at the Barcelona Science Park (University of Barcelona) under the supervision of Professors Fernando Albericio and Mercedes Álvarez. His Ph.D. work involved the development of new protecting groups for the synthesis of complex peptides. In October 2008, he moved to Dr. David R. Spring's group at the University of Cambridge (U.K.) where he is currently a postdoctoral fellow working in Diversity Oriented Synthesis of small molecules as antibiotics. His research interests include the development of new methodologies for solid- and solution-phase peptide synthesis and the research for new bioactive compounds.

Mercedes Álvarez received her Ph.D. in Chemistry from the University of Barcelona under the supervision of Prof. Ricardo Granados. She has a permanent position in the University of Barcelona as Associate Professor in the Faculty of Pharmacy. In 1990, she spent a sabbatical year in The Manchester University working with Prof. John A. Joule. After that period, she started a long collaboration between Manchester and Barcelona Universities for developing new procedures for the synthesis of marine natural products with polyheterocyclic structure and biological activities. In 2002, she was invited to joint with the group led by Prof. Fernando Albericio and to move her research group to the Science Parc of Barcelona. Currently, she holds a double appointment as Professor at the University of Barcelona and Researcher at the Barcelona Biomedical Research Institute in the Barcelona Science Park. Her major research interests cover synthesis of natural products, heterocyclic chemistry, combinatorial chemistry, and solid-phase methodology, as well as synthesis of small molecules with therapeutic activities.

Orthogonal protection schemes are usually milder because selective deprotection is governed by alternative cleavage mechanisms rather than by reaction rates. Since the pioneernig work of Bergmann and Zervas, the development of new protecting groups has been deeply tied to peptide chemistry. Protection is totally mandatory for the construction of these polyfunctional molecules, which contain up to eight distinct functional groups in addition to indole and imidazole rings, which should also be protected. Only the carbonyl function is absent from the natural amino acids, because even phosphate-protecting groups have been developed for the synthesis of phosphopeptides. Thus, the protecting groups first developed for peptide synthesis have been rapidily adapted for the protection of building blocks used for the contruction of nonpeptide molecules.[1,2]

Herein, we provide a concise but deep analysis of the protection of amino acids. The review is divided into sections depending on the amino acid funcionalities protected. For each case, methods for the introduction of the protecting groups as well as for their removal are discussed. In each section, protecting groups are classified based on the following criteria: (i) the most used in a Boc/Bn strategy; (ii) the most used in a Fmoc/^{t}Bu strategy; (iii) decreased order of lability; and (iv) the most recently described, for which, in most cases, their potential has not yet been explored. In all cases, families of protecting groups are classified together. The compatibility of each protecting group with regard to the others is indicated in the column "stability to the removal of", which shows which of the following α-amino-protecting groups (Boc, Fmoc, Z, Trt, Alloc, and *p*NZ) can be removed without affecting a particular protector.

Special attention has been given to new protecting groups described in 2000−2008. Those described in the literature earlier and those that not have found a broad use have been omitted from this review.

2. α-Amino

2.1. General

Protection of the α-amino functionality of amino acids is one of the most important issues in peptide chemistry and is mandatory to prevent polymerization of the amino acid once it is activated.

Because most peptide syntheses, both in solution and on solid phase, are carried out in the *C* to *N* direction, α-amino-protecting groups (temporary protecting groups) are removed several times during the synthesis, and therefore, removal must be done in mild conditions that do not affect the remaining protecting groups (permanent, usually removed in the last step of the synthetic process, and semipermanent, usually at the *C*-terminus, removed in the presence of all other protecting groups, when the peptide is to be coupled at its *C*-terminus) or even the peptidic chain.

The α-amino-protecting group should confer solubility in the most common solvents and prevent or minimize epimerization during the coupling, and its removal should be fast, efficient, and free of side reactions and should render easily eliminated byproducts. Other desired characteristics of α-amino-protected amino acids are that they are crystalline solids, thereby facilitating manipulation, and stable enough.

The most common α-amino-protecting groups for solid-phase peptide synthesis (SPPS) are the 9-fluorenylmethoxycarbonyl (Fmoc) and the *tert*-butyloxycarbonyl (Boc) groups, used in the Fmoc/*tert*-butyl (^{t}Bu) and Boc/benzyl (Bn) strategies, respectively.

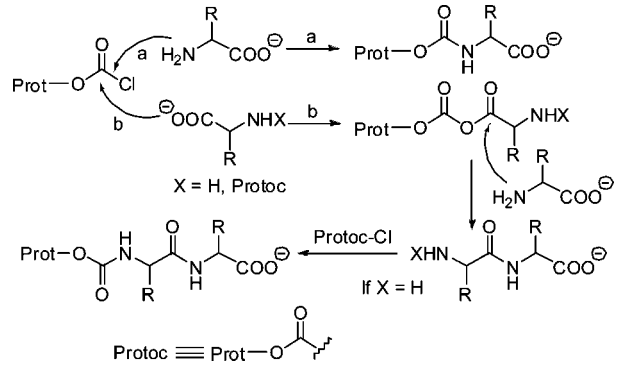


Figure 1. Mechanism for the formation of protected dipeptides during the protection of amino acids with haloformates. Adapted with permission from ref 20. Copyright 2007 Wiley-Blackwell.

For solution synthesis, other α-amino-protecting groups used are the Z, the Nps (2-nitrophenylsulfenyl), and the Bpoc [2-(4-biphenyl)isopropoxycarbonyl] in combination with *t*Bu-type side-chain protection, or the Boc group in combination with Bn-type side-chain protection.

2.2. Introduction of the Protecting Groups

Because there are several types of α-amino-protecting groups, there is a wide range of protection methodologies. Most of these are based on the reaction of the free amino acids (side-chain-protected if necessary; see ω-amino protection part for selective Lys and Orn side-chain protection), with a haloformate[7] or dicarbonate[8,9] of the protecting group under Schotten Baumann conditions (use of biphasic system: organic solvent−aqueous basic conditions)[10] or with the corresponding halide in organic solvents.[11] Nevertheless, in some cases, the presence of the free α-carboxylic acid can interfere in the reaction and lead, for instance, to the formation of dipeptides (Figure 1).[12−19]

The methodologies used to overcome this problem can be divided into two types: those that involve a carboxylic acid-protecting group that is removed upon amino protection and those that involve less-reactive electrophiles on the reagent used to introduce the protecting group. An example of the former is the use of trimethylsilyl esters of amino acids prepared in situ,[19,21] while an illustration of the latter is the use of *N*-hydroxysuccinimido (HOSu) derivative or the corresponding azide, as in the case of the introduction of Fmoc where Fmoc-OSu or Fmoc-N_3 are used instead of Fmoc-Cl. However, the use of Fmoc-OSu can lead to the formation of tiny amounts of Fmoc-β-Ala-OH or even of Fmoc- β-Ala-AA-OH (Figure 2), which can jeopardize the preparation of Fmoc-amino acids for the production of peptide-based active pharmaceutical ingredients (API).[20,22]

2.3. Removal

2.3.1. Protecting Groups Removed by Acid (Table 1)

***tert*-Butyloxycarbonyl (Boc).**[23,24] Boc-amino acids are generally crystalline solids, and their particular suitability for SPPS has been clearly demonstrated.[25,26] The Boc group has been used for the solid-phase synthesis (SPS) of a number of relevant peptides using the so-called Boc/Bn strategy. The most common removal conditions for Boc are 25−50% TFA in DCM, but other acids, such as 1 M trimethylsilyl chloride

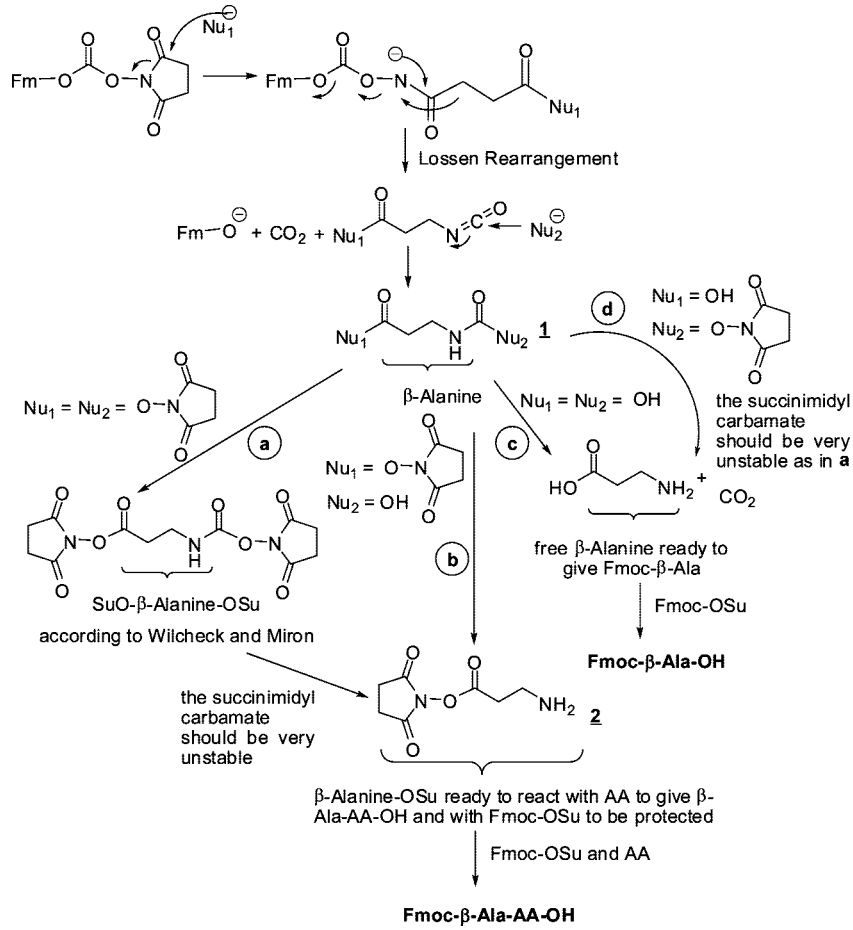


Figure 2. Mechanism for the formation of Fmoc-*β*-Ala-OH and Fmoc-*β*-Ala-AA-OH during the protection of amino acids. Adapted with permission from ref 20. Copyright 2007 Wiley-Blackwell.

(TMS-Cl) phenol in DCM,[27] 4 M HCl in dioxane, and 2 M $MeSO_3H$ in dioxane,[28] have been successfully used for solution and solid-phase synthetic strategies. The Boc group is stable to bases and nucleophiles as well as to catalytic hydrogenation.

Trityl (Trt).[29,30] It is removed with 1% TFA in DCM or 0.1 M HOBt in 2,2,2-trifluoroethanol (TFE) in solution. It can be removed in even milder conditions such as 0.2% TFA, 1% H_2O in DCM,[31] or 3% trichloroacetic acid (TCA) in DCM,[32] which are compatible with the TFA labile 3-(4-hydroxymethylphenoxy)propionic acid (AB) linker or even with the more acid labile Riniker handle,[33] as well as with the synthesis of oligonucleotide−peptide conjugates. Coupling yields of Trt-amino acids are lower than those of carbamate-protected amino acids. An important application of the Trt group is for the protection of the second *C*-terminal amino acid in order to prevent diketopiperazines (DKPs) formation in a similar way as for the Boc strategy.[34,35] This procedure involves the coupling of the third amino acid with in situ neutralization after the removal of the Trt group.[31]

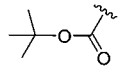
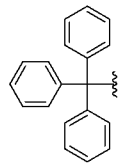
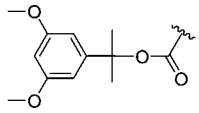
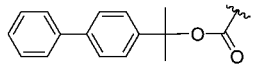
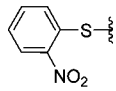
Incorporation of Trt-amino acids is more difficult than that of carbamate-protected amino acids, which implies the use of more powerful activating conditions. However, the bulkiness of the Trt group protects the α-proton from the base abstraction and, therefore, makes Trt-AA-OH more difficult to racemize.[36]

α,α-Dimethyl-3,5-dimethoxybenzyloxycarbonyl (Ddz).[37] Although Ddz is more acid-stable than the Bpoc and the Trt groups, its removal with 1−5% TFA in DCM makes it compatible with ^{t}Bu-type side-chain protection.[38] It can also be removed by photolysis at wavelengths above 280 nm,[37] which makes it potentially very useful for SPS library-screening procedures. It has been used to prevent DKP formation in the backbone amide linker (BAL) strategy in a similar way as the Trt group.[39] However, an advantage of Ddz- over Trt-amino acids is that their incorporation is easier, which is a crucial factor when the corresponding amino acids are to be incorporated on hindered amines.[39]

2-(4-Biphenyl)isopropoxycarbonyl (Bpoc).[40] It is a highly acid-sensitive carbamate-type protecting group, which is removed with 0.2−0.5% of TFA except when used in poly(ethylene glycol)-based resins, in which more TFA is required because some of the acid is used to protonate the oxymethyl moieties.[41] This is a common characteristic of several acid labile-protecting groups.[42] Most Bpoc-amino acids are oils and are unstable because the free α-carboxylic acid is acidic enough to remove the Bpoc group. Thus, these amino acids are usually stored either as DCHA salts or as pentafluorophenyl esters.[43] In the early stages of SPPS, before the introduction of the Fmoc group, Bpoc-amino acids have been used in combination with ^{t}Bu-type sid- chain protection.[41] Currently, Bpoc-amino acids are used mostly for peptide derivatives containing phosphate groups such as phosphopeptides or peptide−oligonucleotide conjugates.[44,45]

2-Nitrophenylsulfenyl (Nps).[46] It is removed most conveniently with diluted solutions of HCl in AcOH.[47] It is resistant to bases but can be removed by nucleophiles such as 2-mercaptopyridine in combination with AcOH in MeOH, DMF, or DCM.[48] Removal using a Ni Raney column and organic solvents, such as DMF, has also been described. [49]

Table 1. α-Amino-Protecting Groups Removed by Acid

Name and Structure	Removal conditions	Stability to the removal of	Ref.
tert-Butyloxycarbonyl (Boc) 	1) 25-50% TFA-DCM 2) 4 M HCl in dioxane 3) 2 M $MeSO_3H$ in dioxane 4) 1 M TMS-Cl, 1 M phenol-DCM	Fmoc, Z,[a] Trt, Alloc, *p*NZ	23,24, 25,26, 27,28
Trityl (Trt) 	1) 1% TFA-DCM 2) 0.1 M HOBt-TFE 3) 0.2% TFA, 1% H_2O-DCM 4) 3% TCA-DCM	Fmoc, Alloc	29,30, 31,32, 33,34, 35 36
3,5-Dimethoxyphenylisoproxycarbonyl (Ddz) 	1-5% TFA-DCM	Fmoc, Alloc	37,38, 39
2-(4-Biphenyl)isopropoxycarbonyl (Bpoc) 	0.2-0.5%-TFA	Fmoc, Alloc	40,41, 42,43, 44,45
2-Nitrophenylsulfenyl (Nps) 	1) Diluted solutions of HCl-$CHCl_3$-AcOH 2) 2-Mercaptopyridine-AcOH-MeOH, DMF or DCM 3) Ni Raney column in DMF	Fmoc	46,47, 48,49

[a] Catalytic hydrogenation removal.

Nps has been applied in both solution and SPS. Its high acid lability requires similar precautions to the Bpoc group in the presence of the free α-carboxylic acid.

Benzyloxycarbonyl (Z). See section on "other protecting groups".

2.3.2. Protecting Groups Removed by Base (Table 2)

9-Fluorenylmethoxycarbonyl (Fmoc).[50,51] It is removed by bases (mainly secondary amines, because they are better at capturing the dibenzofulvene generated during the removal) and is stable to acids. It is not completely stable to the catalytic hydrogenolysis treatment required to remove benzyl esters when Pd/C or PtO_2 are used as catalysts. The most selective catalyst is $Pd/BaSO_4$.[52] Solution removal is done by liquid NH_3 (10 h) and morpholine or piperidine (within minutes), 10% diethylamine (DEA), dimethylacetamide (DMA) (2 h),[53] and polymeric (silica gel or polystyrene) secondary amines (i.e., piperazine, piperidine) in organic solvents.[54,55] This was applied for the first time for SPPS by two different laboratories independently.[56,57] Since then, several optimized removal conditions for SPS have been described, with the most relevant being 20% piperidine in DMF,[56] which is the most common; 1−5% DBU in DMF;[58,59] morpholine−DMF (1:1)[60] or 2% HOBt; 2% hexamethyleneimine; and 25% *N*-methylpyrrolidine in DMSO−NMP (1:1),[61] with the latter method leaving thioesters intact. The addition of a relatively small amount of HOBt to the piperidine solution [0.1 M HOBt in piperidine−DMF (2:8)] reduces the formation of aspartimide in the sequences prone to this side reaction.[62,63]

Fmoc α-amino protection has been used for the SPS of several relevant peptides using the so-called Fmoc/^{t}Bu strategy, with the production in Tm scale of the T20 peptide being one of the most important examples.[64] Nevertheless, the low solubility of some Fmoc derivatives in the most commonly used solvents for SPPS has stimulated the search for new base-labile protecting groups.

2-(4-Nitrophenylsulfonyl)ethoxycarbonyl (Nsc).[65] This is considered the most promising alternative to the Fmoc group.[66−69] Nsc-amino acids are crystalline solids, more

soluble in common solvents than Fmoc amino acids, and can be deprotected with 20% of piperidine or 1% DBU in DMF or preferably in DMF−dioxane (1:1).[65,67] Nevertheless, the use of DBU accelerates aspartimide formation and other side reactions.[70] Nsc is 3−10 times more base-stable than the Fmoc group,[67] thereby preventing its undesired removal under slightly basic conditions. This is particularly relevant in the synthesis of polyproline peptides in which the use of the Fmoc group leads to deletions caused by premature Fmoc removal by the secondary amine of Pro, whereas no Pro insertions are observed when Nsc is used.[68] Nsc is also important in automated SPS, where amino acid solutions are stored for a long time. Further advantages of the Nsc group versus the Fmoc group are that the formation of the olefin−amine adduct after removal is irreversible and faster for Nsc[67] and Nsc protection reduces racemization compared to Fmoc protection,[68] which is particularly important in *C*-terminal Ser, Cys, and His.

(1,1-Dioxobenzo[*b*]thiophene-2-yl)methyloxycarbonyl (Bsmoc).[71] It is the most important of a series of protecting groups that are removed via a Michael addition. Other protecting groups from the same family are the Bspoc (2-*tert*-butylsulfonyl-2-propenoxycarbonyl)[72] and the Mspoc (2-methylsulfonyl-3-phenyl-1-prop-2-enyloxycarbonyl)[73] and the Mspoc groups. The Michael addition removal mechanism has several advantages over the β-elimination removal mechanism of Fmoc and Nsc: (i) back-alkylation by the β-elimination byproduct is prevented because the deblocking event is also a scavenging event;[71] (ii) base-catalyzed side reactions, such as aspartimide formation, are minimized as a result of lower concentrations of secondary amines;[71,74] and (iii) the method can be applied to the rapid, continuous solution-synthesis technique.[74,75] Bsmoc-amino acids have been used to synthesize several model peptides in which the Bsmoc group was removed with 2−5% piperidine in DMF[71] and have shown better performance than Fmoc-amino acids in difficult couplings such as Aib-Aib.[42] Furthermore, the Bsmoc group can be selectively removed with 2% of tris(2-aminoethyl)amine (TAEA) in DCM in the presence of Fm esters.[71]

(1,1-Dioxonaphtho[1,2-*b*]thiophene-2-yl)methyloxycarbonyl (α-Nsmoc).[76] It is a novel alternative to the Bsmoc group and is removed in the same way but slightly faster. α-Nsmoc-amino acids are crystalline solids; thus, they are a good alternative to Bsmoc in the cases where Bsmoc-amino acids are oils.

(1-(4,4-Dimethyl-2,6-dioxocyclohex-1-ylidene)-3-ethyl) (Dde) and 1-(4,4-Dimethyl-2,6-dioxocyclohex-1-ylidene)-3-methylbutyl (ivDde). Both groups are removed by hydranzinolysis; although they can be used for α-amino protection,[77] their principal application is for the protection of Lys and Orn side chains (see the section on Lys and Orn protection).

2,7-Di-*tert*-butyl-Fmoc (Fmoc*).[78] It is removed in the same conditions as the Fmoc group but is up to four times slower. Fmoc*-amino acid derivatives are more soluble than the Fmoc ones.[78,79] They have been recently used for the synthesis of cyclic modular β-sheets.[80]

2-Fluoro-Fmoc (Fmoc(2F)).[81] It is a more base-labile derivative of the Fmoc group and has been used for the SPS of phosphopeptide thioesters. It is removed with a 4 min treatment with 4% HOBt in 1-methylpyrrolidine−hexamethylenimine−NMP(1-methylpyrrolidin-2-one)−DMSO (25:2:50:50).

2-Monoisooctyl-Fmoc (mio-Fmoc) and 2,7-Diisooctyl-Fmoc (dio-Fmoc).[82] Both are novel protecting groups reported to show greater solubility than Fmoc* derivatives in DCM−MeOH (100:4). Their removal with 20% piperidine in DMF is slower than Fmoc removal: 2 times slower in the case of mio-Fmoc and 5 times slower for dio-Fmoc.

Tetrachlorophthaloyl (TCP).[83] It is a relatively new protecting group proposed for SPPS. It is removed with hydrazine in DMF (15% of hydrazine, at 40 °C, 1 h for repetitive deprotection) but stable to piperidine and to Boc removal conditions. It is also used for side-chain amino protection.

2-[Phenyl(methyl)sulfonio]ethyloxycarbonyl tetrafluoroborate (Pms).[84] Pms-amino acids are water-soluble. They have been developed relatively recently and allow SPPS in water. Pms is removed with 5% aqueous $NaHCO_3$, 2 × 3 min and 1 × 30 min for SPS.[84,85] Nevertheless, since Pms is an onium salt, it is rather unstable compared to conventional protecting groups.[86]

Ethanesulfonylethoxycarbonyl (Esc).[86] It is another relatively new protecting group for peptide synthesis in water. The derivatives of Esc are more stable than those of Pms. It is removed either by 0.025 M NaOH in H_2O−EtOH (1:1) or 0.05 M TBAF in DMF.

2-(4-Sulfophenylsulfonyl)ethoxycarbonyl (Sps).[87] Developed parallel to Esc at almost the same time, it is also a protecting group for SPS in water. It is removed with 5% aqueous Na_2CO_3. Sps-amino acids have a similar stability to Esc ones, but with the advantage that they absorb in the UV.

2.3.3. Other Protecting Groups (Table 3)

Benzyloxycarbonyl (Z).[4] It is one of the most widely used α-amino-protecting groups for peptide synthesis in solution because of (i) the easy preparation of *Z*-protected amino acids; (ii) the high stability of protected amino acids and peptides, which are stable to base and mild acid treatments (stability to Boc removal); (iii) the versatile removal conditions: by catalytic hydrogenolysis during chain elongation or by strong acids (HBr in acetic acid,[88] TFA at high temperatures,[89] TFA−thioanisole,[90] liquid HF,[91] BBr_3)[92] in the final deprotection of the peptide; and (iv) the supression of racemization during peptide-bond formation.[93]

Allyloxycarbonyl (Alloc).[94−98] It is removed by a palladium-catalyzed (usually $Pd(PPh_3)_4$) transfer of the allyl unit to various nucleophiles/scavengers (preferably $H_3N{\cdot}BH_3$, $Me_2NH{\cdot}BH_3$, or $PhSiH_3$)[99,100] in the presence of a proton source. The use of scavengers is mandatory to prevent allylation of the free amine upon Alloc removal. If removed on solid phase, washings with sodium *N*,*N*-diethyldithiocarbamate (0.02 M in DMF, 3 × 15 min) are carried out in order to remove Pd. Alloc-amino acids are oils but can be stored as DCHA salts or pentafluorophenyl esters, both of which are crystalline solids.[101] The use of Alloc group is compatible with the Boc/Bn and Fmoc/^{t}Bu strategies and allows tandem removal−acylation reactions when the palladium-catalyzed amino deblocking is performed in the presence of acylating agents.[102] This strategy has been used to prevent DKP formation.[103] Alloc has recently been applied as an α-amino-protecting group for a convergent synthesis of the antitumoral peptide Kahalalide F.[104]

***o*-Nitrobenzenesulfonyl (*o*NBS) and *p*-nitrobenzenesulfonyl (*p*NBS).[105]** The most used is *o*NBS. It is removed by a nucleophilic aromatic substitution mechanism using β-mer-

Table 2. α-Amino-Protecting Groups Removed by Base

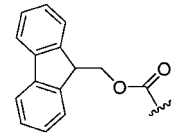
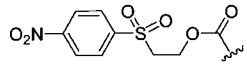
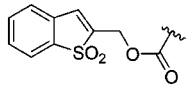
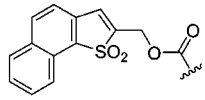
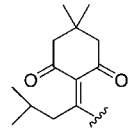
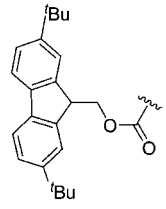
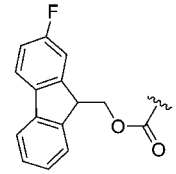
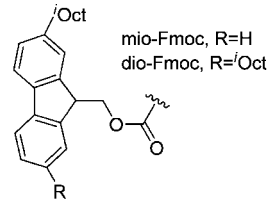
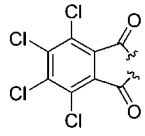
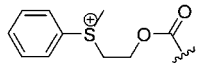
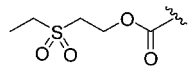
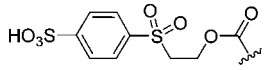
Name and Structure	Removal conditions	Stability to the removal of	Ref.
9-Fluorenylmethoxycarbonyl (Fmoc) 	Solid phase: 1) 20% piperidine-DMF 2) 1-5% DBU-DMF 3) morpholine-DMF (1:1) 4) 2% HOBt, 2% hexamethyleneimine, 25% *N*-methylpyrrolidine in DMSO-NMP (1:1) Solution: 1) NH_3 (10 h) 2) morpholine or piperidine in organic solvents (within minutes) 3) 10% DEA,DMA (2 h) 4) polymeric secondary amines (i.e. piperidine, piperazines) in organic solvents	Boc, Z,[a] Trt, Alloc, *p*NZ [a]	50,51, 52,53, 54,55, 56,57, 58,59, 60,61, 62,63, 64
2-(4-Nitrophenylsulfonyl) ethoxycarbonyl (Nsc) 	1) 20% of piperidine-DMF or DMF-dioxane (1:1) 2) 1% DBU-DMF or DMF-dioxane (1:1)	Boc, Trt, Alloc	65,66, 67,68, 69,70
(1,1-Dioxobenzo[b]thiophene-2-yl)methyloxycarbonyl (Bsmoc) 	1) 2-5% piperidine-DMF 2) 2% TAEA-DCM	Boc, Trt, Alloc	42,71,72, 73,74,75
(1,1-Dioxonaphtho[1,2-b]thiophene-2-yl)methyloxycarbonyl (α-Nsmoc) 	1) 2-5% piperidine-DMF 2) 2% TAEA-DCM	Boc, Trt, Alloc	76
1-(4,4-Dimethyl-2,6-dioxocyclohex-1-ylidene)-3-methylbutyl (ivDde) 	2% $N_2H_4 \cdot H_2O$-DMF	Boc, Fmoc, Z,[a] Trt, Alloc	77
2,7-Di-tert-butyl-Fmoc (Fmoc*) 	20% piperidine-DMF (solid phase)	Boc, Trt, Alloc	78,79,80

Table 2. (Continued)

Name and Structure	Removal conditions	Stability to the removal of	Ref.
2-Fluoro-Fmoc (Fmoc(2F)) 	4% HOBt-1-methylpyrrolidine-hexamethylenimine-NMP-DMSO (25:2:50:50), 4 min.	Boc, Trt, Alloc	81
2-Monoisooctyl-Fmoc (mio-Fmoc) and 2,7-Diisooctyl-Fmoc (dio-Fmoc) 	20% piperidine-DMF		82
Tetrachlorophthaloyl (TCP) 	15% hydrazine-DMF, 1 h, 40°C	Boc, Fmoc, Trt	83
2-[Phenyl(methyl)sulfonio])ethyloxy carbonyl tetrafluoroborate (Pms) 	5% $NaHCO_{3\,(aq)}$	Boc, Trt	84,85, 86
Ethanesulfonylethoxycarbonyl (Esc) 	0.025 M NaOH-H_2O-EtOH (1:1)	Boc, Trt	86
2-(4-Sulfophenylsulfonyl)ethoxy carbonyl (Sps) 	5% $Na_2CO_{3\,(aq)}$	Boc, Trt	87

[a] Except catalytic hydrogenation removal.

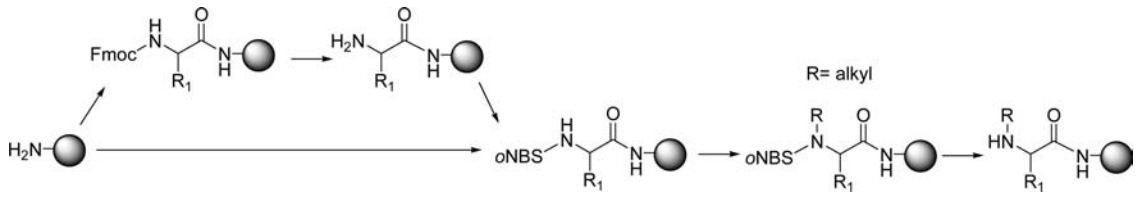


Figure 3. *o*NBS protection for the synthesis of *N*-alkyl peptides. Reprinted with permission from refs 107 and 108. Copyright 1997 and 2005 American Chemical Society.

captoethanol and DBU when it is protecting *N*-alkyl derivatives, but the deblocking of primary amines fails under these conditions and the cocktail used is 5% thiophenol in DMF containing 2 equiv of K_2CO_3. The main advantage of *o*NBS- versus Fmoc-amino acids is that the former do not form oxazolones and thus *o*NBS-amino acyl chlorides can be used in difficult couplings with less risk of racemization.[106] *o*NBS α-amino-protection is also used for site-specific alkylation of amino acids on solid phase,[107,108] making these groups unique for the preparation of *N*-Me peptides (Figure 3).

2,4-Dinitrobenzenesulfonyl (dNBS).[109] It is removed by treatment with $HSCH_2CO_2H$ (1.2 equiv) and TEA (3 equiv) in DCM for 30 min, leaving *o*NBS unaltered.

Benzothiazole-2-sulfonyl (Bts).[106,110] This is used in solution in a similar way to NBS groups. It is removed using thiophenol and base (K_2CO_3, DIPEA, or ^{t}BuOK) in both primary and secondary amines, $NaBH_4$ in EtOH[42] or $HS(CH_2)_2CO_2H$, Na_2CO_3 in DMF[111] for secondary amines, and other reducing agents, such as Zn, H_3PO_2, Al/Hg,[106] which can be used for primary and secondary amines. However, in the latter case, the reaction is slower and highly concentration-dependent. Bts has been used for the synthesis of the cyclosporin 8−11 tetrapeptide subunit, which contains three *N*-methylamino acids,[110] and more recently for the synthesis of macrocyclic antagonists of the Human Motilin Receptor.[112]

2-Nitrophenylsulfanyl (Nps). See the section on protecting groups removed by acid.

2,2,2-Trichloroethyloxycarbonyl (Troc).[113] It is a classical protecting group that can be removed selectively in the presence of Z, Boc, Fmoc, and Alloc groups via a Grob fragmentation using Zn dust in 90% aqueous AcOH or other reducing agents.[113,114] It is not stable to catalytic hydrogenolysis.

Dithiasuccinoyl (Dts).[115] It is removed with mild thiolysis using 0.5 M β-mercaptoethanol and 0.5 M DIPEA in DCM or 0.5 M *N*-methylmercaptoacetamide (NMM) in DCM.[116] It was used for α-amino protection in the first *three-dimensional* orthogonal protection scheme suitable for the preparation of fully and partially protected peptides, which also involved *tert*-butyl type groups for side-chain protection and an *o*-nitrobenzyl ester linkage.[6] Although Dts is not commonly used for the synthesis of peptides, it has proved useful for the synthesis of peptide nucleic acids (PNA)[117] and *O*-glycopeptides by protecting the 2-amino substituent in the corresponding glycosyl donors.[118]

***p*-Nitrobenzyloxycarbonyl (*p*NZ).**[119] It is a classical protecting group that has recently found further applicability for the synthesis of complex peptides as well as for minimizing side reactions.[120] It is much more stable to strong acids than the Z group and is removed by reduction with tin(II) chloride in nearly neutral conditions (1.6 mM $HCl_{(dioxane)}$) in solid-phase and in solution synthesis,[120,104] as well as by catalytic hydrogenolysis or $Na_2S_2O_4$[121] for solution synthesis. *p*NZ is orthogonal to the three most important amino-protecting groups, Boc, Fmoc, and Alloc, thereby making it highly suitable for the synthesis of cyclic complex peptides such as oxathiocoraline.[122] If the second *C*-terminal amino acid in SPPS is introduced as a *p*NZ derivative and the *p*NZ group is removed using $SnCl_2$ and catalytic ammounts of HCl, the formation of DKP is prevented. The formation of aspartimides is also prevented using *p*NZ-amino acids from the Asp residue to the *N*-terminus.[120]

α-Azido Carboxylic Acids.[122,123] Although not widely used because of the instability of azides, there are examples of their successful application in SPPS.[125,126] The azide is reduced to amine using trimethylphosphine in dioxane. α-Azido carboxylic acids can be coupled as acyl chlorides without oxazolone formation.

Propargyloxycarbonyl (Poc).[127,128] It is removed by ultrasonic irradiation in the presence of tetrathiomolybdate complexes such as $[(PhCH_2NEt_3)_2MoS_4]$ in AcCN. It is a relatively new and still not widely used protecting group for solution-phase peptide synthesis. It is stable to Boc removal conditions and has been used to protect amino acid chlorides to be used in couplings on hindered amines without racemization.

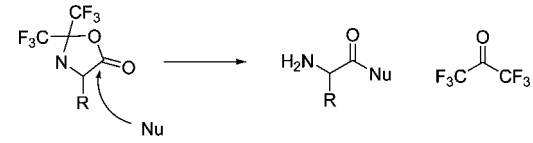


Figure 4. Deprotection of a HFA-protected amino acid via nucleophilic attack.

***o*-Nitrobenzyloxycarbonyl (*o*NZ) and 6-Nitroveratryloxycarbonyl (NVOC).**[129] They are removed by photolysis at wavelengths greater than 320 nm in the presence of additives such as N_2H_4, $NH_2OH\cdot HCl$, or semicarbazide hydrochloride for several hours, with *o*NZ being the most easily removed. NVOC has been used for combinatorial library production using the Affymax methodology.[130] Research effort is being made to develop more easily removable photolabile protecting groups.

2-(2-Nitrophenyl)propyloxycarbonyl (NPPOC).[131] It is a photolabile amino-protecting group that is removed by UV light ($\lambda = 365$ nm) about twice as fast as the classical NVOC group.

2-(3,4-Methylenedioxy-6-nitrophenyl)propyloxycarbonyl (MNPPOC).[132] It is removed faster than the NPPOC and has been developed recently by the same research group.

Ninhydrin (Nin). See the section on Cys protection.

9-(4-Bromophenyl)-9-fluorenyl (BrPhF).[133] It is a recently proposed safety-catch amino-protecting group and has been tested only for solution synthesis. It prevents epimerization and is more acid-stable than the Trt group because of the antiaromatic nature of the fluorenyl group. ^{t}Bu esters can be selectively cleaved in its presence by using $ZnBr_2$ in DCM or trichloroacetic acid.[134,133] BrPhF is removed by Pd-catalyzed aminolysis with morpholine, followed by treatment of the resulting acid-labile morpholine adduct with DCA and triethylsilane (TES) in DCM.

Azidomethoxycarbonyl (Azoc).[135] It is a novel protecting group proposed for solution and solid-phase synthesis. It is removed by reduction of the azide with phosphines. The removal is rapid when PMe_3 or PBu_3 (5 min on solid phase) are used and slower with polymer-bound PPh_3 (30 min). Azoc is orthogonal to Fmoc and Mtt.

Bidentate Protecting Groups.[136] Another possibility is the use of bidentate reagents such as *N*-carboxyanhydrides (NCA) and the oxazolidinones derived from hexafluoroacetone (HFA) or formaldehyde, which undergo heterocyclization with the amino and the α-carboxylic groups. In the heterocycle, the carboxylic group is electrophilic, and a carboxy-derivatization is accompanied by *N*-deprotection (Figure 4).

3. Lysine (Lys), Ornithine (Orn), Diaminopropionic Acid (Dap), and Diaminobutyric Acid (Dab)

3.1. General

The protection of the side chains of lysine (Lys) and ornithine (Orn) as well as diaminopropionc acid (Dap) and diaminobutyric acid (Dab) (Figure 5) is essential in peptide synthesis to prevent their acylation, which would lead to the formation of undesired branched peptides.

Several groups used for the α-amino funcionality have found application for amino side-chain protection. It is worth

Table 3. Other α-Amino-Protecting Groups

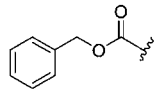
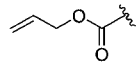
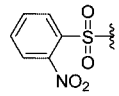
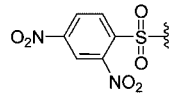
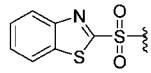
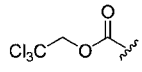
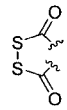
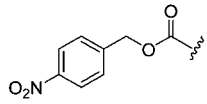
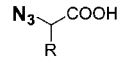
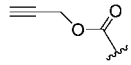
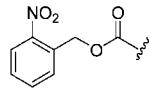
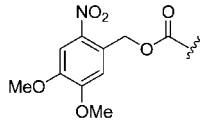
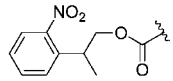
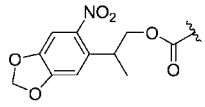
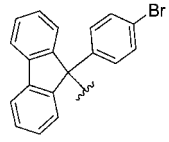
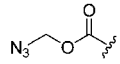
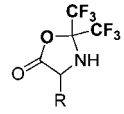
Name and Structure	Removal conditions	Stability to the removal of	Ref.
Benzyloxycarbonyl (Z) 	1) H_2 cat 2) Strong acids such as: HBr in AcOH, TFA at high temperatures, TFA-thioanisole or liquid HF 3) BBr_3	Boc, Fmoc, Trt, Alloc, *p*NZ [a]	4,88,89,90, 91,92,93
Allyloxycarbonyl (Alloc) 	$Pd(PPh)_3$ cat., scavengers: $H_3N{\cdot}BH_3$, $Me_2NH{\cdot}BH_3$ or $PhSiH_3$ in organic solvents	Boc, Fmoc, Trt, *p*NZ [a]	94,95,96, 97,98,99, 100,101,102, 103,104
o-Nitrobenzenesulfonyl (oNBS) 	1) 5% thiophenol-DMF, 2 eq. of K_2CO_3 (primary amines) 2) β-mercaptoethanol and DBU (secondary amines)	Boc, Fmoc, Trt	105,106, 107,108
2,4-Dinitrobenzenesulfonyl (dNBS) 	$HSCH_2CO_2H$ (1.2 eq.), TEA (3 eq.) in DCM	Boc, Trt	109
Benzothiazole-2-sulfonyl (Bts) 	1) Al/Hg 2) Zn 3) H_3PO_2 4) PhSH and base (K_2CO_3, DIPEA, potassium *tert*-butoxyde) 5) $NaBH_4$ in EtOH	-	42,106,110, 111,112
2,2,2-Trichloroethyloxycarbonyl (Troc) 	Zn in 90% $AcOH_{(aq)}$	Boc, Fmoc, Trt	113,114
Dithiasuccinoyl (Dts) 	1) 0.5 M β-mercaptoethanol and 0.5 M DIPEA-DCM 2) 0.5 M *N*-methylmercaptoacetamide-NMM-DCM	Boc, Trt	6,115,116, 117,118
p-Nitrobenzyloxycarbonyl (pNZ) 	1) 1-6 M $SnCl_2$, 1.6 mM $HCl_{(dioxane)}$ in DMF 2) H_2 cat	Boc, Fmoc, Trt, Alloc	104,119,120, 121,122

Table 3. (Continued)

Name and Structure	Removal conditions	Stability to the removal of	Ref.
α-Azidoacids 	PMe_3 in dioxane	-	123,124,125, 126
Propargyloxycarbonyl (Poc) 	$[(PhCH_2NEt_3)_2MoS_4]$ in AcCN (ultrasonic irradiation)	Boc	127,128
o-Nitrobenzyloxycarbonyl (oNZ) 	photolysis (λ>320 nm), additives:N_2H_4, NH_2OH·HCl, or semicarbazide·HCl (several hours)	Boc, Fmoc, Trt, Alloc	129
4-Nitroveratryloxycarbonyl (NVOC) 	photolysis (λ>320 nm), additives: N_2H_4, NH_2OH·HCl, or semicarbazide·HCl (several hours)	Boc, Fmoc, Trt, Alloc	129,130
2-(2-Nitrophenyl)propyloxycarbonyl (NPPOC) 	photolysis (λ=365 nm), additives: 2.5 mM semicarbazide·HCl in MeOH	Boc, Fmoc, Trt, Alloc	131
2-(3, 4-Methylenedioxy-6-nitrophenyl) propyloxycarbonyl (MNPPOC) 	photolysis ((λ>350 nm))	Boc, Fmoc, Trt, Alloc	132
9-(4-Bromophenyl)-9-fluorenyl (BrPhF) 	i) 2.5 mmol $Pd(OAc)_2$ (0.05 eq.), BINAP (0.05 eq.), dry Cs_2CO_3 (5 eq.), morpholine (1.2 eq.) in toluene at reflux, 24 h. (ii) DCA-TES-DCM (14:3:83), 30 min.	-	133,134
Azidomethoxycarbonyl (Azoc) 	1) 1 M PMe_3 in THF-H_2O (9:1), 2-5 min. 2) 1 M PBu_3 in THF-H_2O (9:1), 2-5 min. 3) Polymer-bound PPh_3 (20 eq.) in THF-H_2O (9:1), 30 min.	Fmoc	135
Hexafluoroacetone (HFA) 	Nucleophiles (i.e. alcohols, amines, H_2O)	Boc, Trt, Alloc[b]	136

[a] Except catalytic hydrogenation removal. [b] Using $PhSiH_3$ as scavenger.

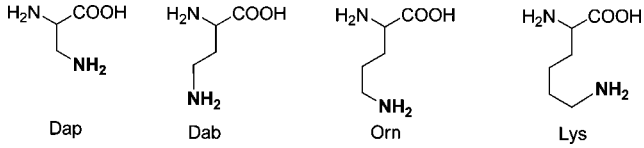


Figure 5. Diaminopropionic acid (Dap), diaminobutyric acid (Dab), ornithine (Orn), and lysine (Lys).

commenting that ω-amino protection is more difficult to remove than α-amino protection because of the higher basicity of the former. Thus, for instance, in the case of trityl-type protection of the α-amino, the Trt group is used, whereas for the ω-amino, the more electron-rich 4-methyltrityl (Mtt) is preferred.

The most used permanent protecting groups for Orn and Lys side chains are the 2-chlorobenzyloxycarbonyl (Cl−Z) and Z groups in the Boc/Bn strategy, as well as the Boc group in the Fmoc/^{t}Bu strategy. In the synthesis of branched or cyclic peptides, there are several protecting groups orthogonal to Boc and Fmoc, with Alloc being among the most popular.

The N^{α}-Fmoc protecting group can be prematurely removed by a primary amine of sufficient basicity, such as the ε-amino group of Lys and to a lesser extent the δ-amino of Orn and the γ-amino of Dab, present in the peptide.[137,138] This side reaction is not promoted by either the β-amino side chain of the Dap residue or the α-amino group. These results are consistent with the pK_a values of these amino functions in the model compounds shown in Table 4. Thus, while the pK_a values of the side amino functions of Lys, Orn, and Dab are very close, the pK_a of Dap is lower by one unit, making this amino function less basic than the other derivatives. The same explanation applies for the α-amino function.

These pK_a values must be taken into consideration when the ω-amino-protecting group of Lys, Orn, or Dab is removed in the presence of an α-amino protected by the Fmoc group. An alternative is a change of strategy, e.g., use of Alloc or Mtt for α-amino protection and Fmoc for ω-amino protection, use of Mtt for ω-amino protection and a coupling/neutralization protocol similar to that used to prevent DKP formation after Mtt removal, or use of Alloc and a tandem deprotection-coupling reaction.[137]

3.2. Introduction of the Protecting Groups

For blocking the α-amino function, a safe method is copper(II) complexation where $CuSO_4 \cdot 5H_2O$ acts as a complexating agent with the α-amino and α-carboxylate groups, thereby allowing the selective protection of the ω-amino funcionality.[139−141] Another alternative also based on complexation is the formation of boron complexes using $B(Et)_3$ as the complexating agent.[142]

In some cases (e.g., Z), side-chain protection can be achieved by protecting both the α-amino and the ω-amino

Table 4. pK_a of Amino Function According To the pKalc Module (PALLAS Version 2.0, CompuDrug)

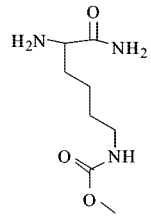
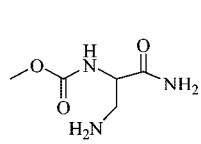
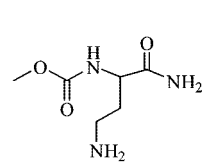
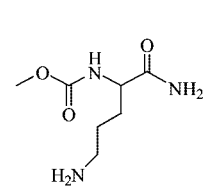
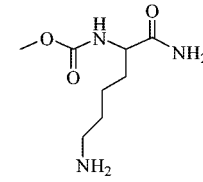
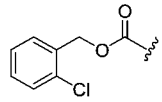
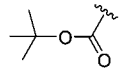
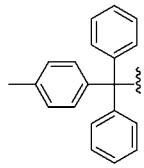
				
*p*Ka: 8.04	*p*Ka: 8.49	*p*Ka: 9.45	*p*Ka: 10.00	*p*Ka: 10.09

Table 5. Lys-, Orn-, Dap-, and Dab-Protecting Groups Removed by Acid

Name and Structure	Removal conditions	Stability to the removal of	Ref.
2-Chlorobenzyloxycarbonyl (Cl-Z) 	1) HF, scavengers 2) TFMSA-TFA 3) H_2 cat.	Boc, Fmoc, Trt, Alloc, *p*NZ [a]	144
tert-Butyloxycarbonyl (Boc) 	25-50% TFA-DCM	Fmoc, Z,[b] Trt, Alloc, *p*NZ	145
4-Methyltrityl (Mtt) 	1) 1% TFA-DCM 2) AcOH-TFE-DCM (1:2:7)	Fmoc, Alloc	146,147

[a] Except catalytic hydrogenation removal. [b] Catalytic hydrogenation removal.

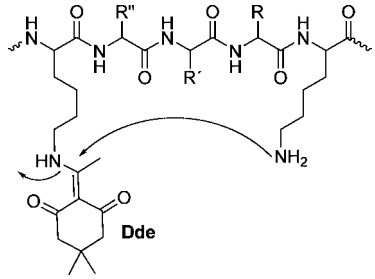


Figure 6. Dde N → N′ migration. This side reaction is prevented using ivDde.

functionalities and then selectively deprotecting the former, taking advantage of their higher lability.[143]

3.3. Removal

3.3.1. Protecting Groups Removed by Acid (Table 5)

2-Chlorobenzyloxycarbonyl (Cl−Z). It is removed with HF or TFMSA and is preferentially used in the Boc/Bzl solid-phase strategy over the Z group because Cl−Z shows major resistance to the repetitive TFA treatments to remove Boc group.[144] Both Z and Cl−Z are stable to bases and can be removed by hydrogenolysis in solution.

***tert*-Butyloxycarbonyl (Boc).** It is removed with 25−50% TFA.[145] It is used in the Fmoc/^{t}Bu solid-phase strategy and is resistant to bases and catalytic hydrogenation.

4-Methyltrityl (Mtt). It can be used for temporary side-chain protection in the Fmoc strategy and is a better option than Boc in the presence of sensitive amino acids such as Tyr, Met, and Trp because it prevents side reactions during TFA cleavage because of the low electrophilicity of the bulky trityl cation. As expected, ω-amino protection with Trt-type groups is more stable than α-amino protection. Removal of Mtt (4-methyltrityl) is performed selectively in the presence of Boc using 1% TFA in DCM for 30 min or with AcOH−TFE−DCM (1:2:7) for 1 h.[146] More acid-labile derivatives, like monomethoxytrityl (Mmt) and dimethoxytrityl (Dmt), are more convenient when hydrophilic resins (e.g., TentaGel) are used.[147]

3.3.2. Protecting Groups Removed by Base (Table 6)

9-Fluorenylmethoxycarbonyl (Fmoc).[142] For additional information, see also the α-amino section. Fmoc is usually removed with 20% of piperidine in DMF or 1−5% DBU in DMF; its stability to acids makes it useful for the synthesis of cyclic and branched peptides using the Boc/Bn strategy. It is not completely stable to catalytic hydrogenation.

1-(4,4-Dimethyl-2,6-dioxocyclohex-1-ylidene)-3-methylbutyl (ivDde).[148] It is useful as a temporary protecting group in the synthesis of cyclic and branched peptides.[149] ivDde is an improved derivative of Dde (1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)-3-ethyl),[150−152] which is considerably less base-labile and, therefore, stable to Fmoc removal conditions and can be removed by hydrazinolysis. An additional advantage of ivDde is that its steric hindrance makes it less prone to migrate to free Lys or Orn side chains (Figure 6).[148] To prevent the reduction of the allyl group by hydrazine, allyl alcohol should be used when ivDde is removed in the presence of allyl-type protecting groups.[153]

Trifluoroacetyl (tfa).[154] It is removed by alkali treatment (0.2 N NaOH in 10 min), [155] aqueous piperidine,[156−158] or sodium borhydride.[159] It is stable to strong acids and, therefore, compatible with the Boc strategy. The basic conditions used for its removal may promote aspartimide formation if aspartic residues are present or pyroglutamyl formation in the case of *N*-terminal glutamine residues.

2-(Methylsulfonyl)ethoxycarbonyl (Msc).[160] It is removed with 0.025−0.5 M $Ba(OH)_2$ or the 4N $NaOH_{(aq)}$−dioxane−MeOH (0.25:7.5:0.25). It is highly stable to acids (TFA, room temperature (rt), and long reaction times; HF, 0 °C, and 30 min; HCl conc, 40 °C, and 1 h)[161] and hydrogenolysis. This reactivity allowed the use of ω-protection with Msc in combination with Boc and Z α-protection.[162]

Tetrachlorophthaloyl (TCP).[163] It is a relatively new protecting group proposed for SPPS and also used for α-amino protection. TCP side-chain protection is removed with ethylenediamine−DMF (1:200) at 40 °C, 1 h, in repetitive deprotections. Nevertheless, hydrazine-based removal used for α-amino deprotection leads to a complex mixture of compounds.[163] TCP is stable to Fmoc, Boc, and Alloc removal conditions.

3.3.3. Other Protecting Groups (Table 7)

Allyloxycarbonyl (Alloc).[164,165,103] It is removed using a palladium catalyst in the presence of a scavenger to capture the generated carbocation. It is compatible with the Boc/Bn and Fmoc/^{t}Bu strategies. See also the section on α-amino protection.

2-Chlorobenzyloxycarbonyl (Cl−Z). See the section on protecting groups removed by acid.

***p*-Nitrobenzyloxycarbonyl (*p*NZ).** See also the α-amino protection section for removal details and references. *p*NZ protection of the side chains of Lys and Orn prevents the undesired removal of the α-Fmoc group after side-chain deprotection.[166,167]

2-Nitrobenzyloxycarbonyl (*o*NZ). See the section on α-amino protection.

6-Nitroveratryloxycarbonyl (NVOC).[168] See the section on α-amino protection.

Phenyldisulfanylethyloxycarbonyl (Phdec) and 2-Pyridyldisulfanylethyloxycarbonyl (Pydec). These are recently developed protecting groups that have been used either for solution or solid-phase synthesis.[169] Both are removed by mild thiolysis using dithiothreitol (DTT) or β-mercaptoethanol in Tris·HCl buffer (pH 8.5−9.0) for deprotection in water or by treatment with β-mercaptoethanol and DBU in NMP for deprotection in an organic medium.

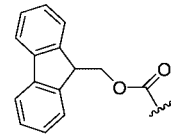
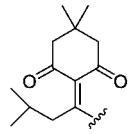
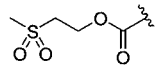
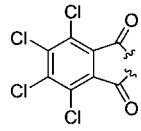
***o*-Nitrobenzenesulfonyl (*o*NBS).** It is widely used for the α-*N*-methylation of amino acids. Because of its high stability to acids and bases, *o*NBS has found application in the side-chain protection of secondary amines derived from Lys and Orn. It is removed from secondary amines by mercaptoethanol in the presence of DBU.[170,171]

4. α-Carboxylic Acid

4.1. General

The protection of the *C*-terminal carboxylic acid is different in SPS to in solution synthesis. In the former, the *C*-terminal is usually linked to the solid support, and therefore, the linker/handle acts as a protecting group. There are excellent reviews covering the linkers/handles used in SPPS, and therefore, they are out of the scope of the present review. Nevertheless, in some synthetic strategies where the peptide is linked to the resin by the backbone by an amino acid side chain, and also in the less-frequent synthesis in the reverse *N*−*C* direction, [39,172,173] *C*-terminal protection is required.

Table 6. Lys-, Orn-, Dap-, and Dab-Protecting Groups Removed by Base

Name and Structure	Removal conditions	Stability to the removal of	Ref.
9-Fluorenylmethoxycarbonyl (Fmoc) 	1) 20% piperidine-DMF 2) 1-5% DBU-DMF (See also α-amino)	Boc, Z,[a] Trt Alloc, *p*NZ[a]	142
1-(4,4-Dimethyl-2,6-dioxocyclohex-1-ylidene)-3-methylbutyl (ivDde) 	2% $N_2H_4 \cdot H_2O$-DMF	Boc, Fmoc, Z,[a] Trt, Alloc	148,149,150, 151,152,153
Trifluoroacetyl (tfa) 	1) 0.2N $NaOH_{(aq)}$ 2) 1 M $piperidine_{(aq)}$ 3) $NaBH_4$ in EtOH	Boc, Z,[a] Trt, Alloc	154,155,156, 157,158,159
2-(Methylsulfonyl)ethoxycarbonyl (Msc) 	1) 0.025-0.5 M $Ba(OH)_2$ 2) 4N $NaOH_{(aq)}$-dioxane-MeOH (0.25:7.5:0.25)	Boc, Z,[b] Trt, Alloc	160,161,162
Tetrachlorophthaloyl (TCP) 	Ethylenediamine-DMF (1:200), 1 h, 40°C	Boc, Fmoc, Trt, Alloc	163

[a] Except catalytic hydrogenation removal. [b] Catalytic hydrogenation removal.

In the case of solution synthesis, *C*-terminal protection is not needed to form the peptide bond. However, in other cases, *C*-terminal protection is mandatory.

4.2. Introduction of the Protecting Groups[174]

Protection of the α-carboxylic acid can be performed mainly by the following methods: (i) reaction of an α-amino-free amino acid with an alcohol in acidic conditions (HCl and *p*-TosOH are the most used acids);[175] (ii) *tert*-butyl protection by reaction of an α-amino-free or protected amino acid with isobutene in acidic conditions (usually *p*-TosOH or H_2SO_4);[176,177] (iii) reaction of an α-amino-protected amino acid in the presence of base or as a cessium salt with the corresponding halide (usually bromide);[178,179] and (iv) reaction of an α-amino-protected amino acid with a condensating agent such as DCC in the presence of DMAP and the alcohol derivative of the protecting group.[180]

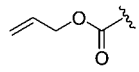
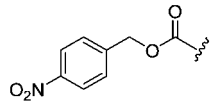
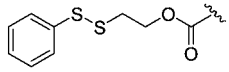
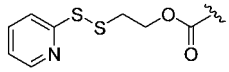
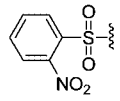
For the particular case of aspartic (Asp) and glutamic (Glu) acids α-carboxyl protection, two main strategies are possible:
• Protection of the α-carboxylic acid after selective protection of the side chain of H-Asp-OH or H-Glu-OH either via acid-catalyzed esterification or in the presence of a copper chelate (see the section on protection of side chain of Asp and Glu). Side-chain deprotection renders the desired protected derivative.[181,182,142]
• Selective protection of the α-carboxylic acid via formation of an intramolecular anhydride between the two carboxylic acids and reaction with the corresponding alcohol or via reaction with a halide in the presence of base. In both cases, *N*-protected Asp or Glu acid are used as starting materials. In the first case, selective α-protection is achieved as a result of the major electrophilicity of the α-carboxylic acid, whereas in the second, the selective protection is due to the major acidity of the α-carboxylic acid. [183,184]

4.3. Removal

4.3.1. Protecting Groups Removed by Acid (Table 8)

***tert*-Butyl (^{t}Bu).[177]** It is used in both solution and solid-phase synthesis. It is removed with high concentrations of TFA (solid phase and solution) or HCl in organic solvents (solution). In the latter case, it is effectively used along with Bpoc N^{α}-protection and Trt side-chain protection or with Z group as N^{α}-protection. It is stable to base-catalyzed hydrolysis, and its bulkiness generally prevents DKP formation.[185]

Table 7. Other Lys-, Orn-, Dap-, and Dab-Protecting Groups

Name and Structure	Removal conditions	Stability to the removal of	Ref.
Allyloxycarbonyl (Alloc) 	$Pd(PPh)_3$ cat., scavengers: $H_3N{\cdot}BH_3$, $Me_2NH{\cdot}BH_3$ or $PhSiH_3$ in organic solvents	Boc, Fmoc, Trt, *p*NZ [a]	103,164,165
p-Nitrobenzyloxycarbonyl (pNZ) 	1) 1-6 M $SnCl_2$, 1.6 mM $HCl_{(dioxane)}$-DMF 2) H_2 cat 3) $Na_2S_2O_4$	Boc, Fmoc, Z,[a] Trt, Alloc	166,167
Phenyldisulphanylethyloxycarbonyl (Phdec) 	1) DTT or β-mercaptoethanol-Tris·HCl buffer (pH 8.5–9.0) 2) β–mercaptoethanol, DBU-NMP	Boc, Fmoc, Trt	169
2-Pyridyldisulphanylethyloxycarbonyl (Pydec) 	1) DTT or β-mercaptoethanol-Tris·HCl buffer (pH 8.5–9.0) 2) β–mercaptoethanol, DBU-NMP	Boc, Fmoc, Trt	169
o-Nitrobenzenesulfonyl (o-NBS) 	β-mercaptoethanol, (5eq.) DBU (10 eq.)-DMF	Boc, Fmoc, Trt	170,171

[a] Except catalytic hydrogenation removal.

Benzyl (Bn). See the section on other protecting groups.

2-Chlorotrityl (2-Cl-Trt).[186] It is removed with 1% TFA in DCM and is used as a semipermanent protecting group for the synthesis of large peptides using a convergent approach.

2,4-Dimethoxybenzyl (Dmb).[187] It is removed with 1% TFA in DCM (6 × 5 min). Because of its high acid lability, it can be removed in the presence of ^{t}Bu-type protecting groups and also on Wang and PAL/Rink resins. It is used for Fmoc/^{t}Bu SPS of "head-to-tail" cyclic peptides.

2-Phenylisopropyl (2-Ph^{i}Pr).[188] It is removed with 4% TFA in DCM for 15 min (Boc group is stable to these conditions).

5-Phenyl-3,4-ethylenedioxythenyl Derivatives (Phenyl-EDOT$_n$).[189] They have been recently developed and are removed using very small concentrations of TFA (0.01−0.5%), with the most acid-labile derivative being the 5-(3,4-dimethoxyphenyl)-3,4-ethylenedioxythenyl.

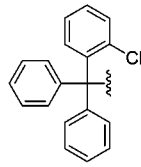
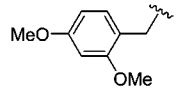
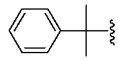
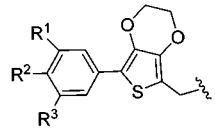
4.3.2. Protecting Groups Removed by Base (Table 9)

9-Fluorenylmethyl (Fm).[190,191] It is removed with secondary amines such as piperidine and DEA in DCM or DMF, as well as by catalytic hydrogenation in solution.[191] Used for SPS in the reverse *N*−*C* direction,[172] as well as for the preparation of "head-to-tail" cyclic peptides.[192]

4-(*N*-[1-(4,4-dimethyl-2,6-dioxocyclohexylidene)-3-methylbutyl]amino)benzyl (Dmab).[193] It is removed by 2% of hydrazine·H_2O−DMF (1:1) within minutes. It is stable to piperidine.

Methyl (Me) and Ethyl (Et).[194] Methyl esters are removed by saponification (usually with LiOH), which can lead to

Table 8. α-Carboxylic Acid-Protecting Groups Removed by Acid

Name and Structure	Removal conditions	Stability to the removal of	Ref.
***tert*-Butyl (^{t}Bu)** 	90% TFA-DCM (solid phase and solution) or 4 M HCl in dioxane (solution)	Fmoc, Z,[a] Trt Alloc, *p*NZ,	177,185
***2*-Chlorotrityl (2-Cl-Trt)** 	1% TFA-DCM	Fmoc, Alloc	186
***2,4*-Dimethoxybenzyl (Dmb)** 	1% TFA-DCM	Fmoc, Alloc	187
***2*-Phenylisopropyl (2-Ph^{i}Pr)** 	4% TFA-DCM	Fmoc, Alloc	188
***5*-Phenyl-3,4-ethylenedioxythenyl (Phenyl-EDOTn)**  $R^1=R^2=R^3=$ OMe; $R^1=R^2=$ OMe, $R^3=$ H; $R^1=R^2=$ H, $R^3=$ OMe or $R^1=R^2=R^3=$ H.	0.01%-0.5% TFA-DCM and scavengers	Fmoc	189

[a] Catalytic hydrogenation removal.

epimerization and degradation of Ser, Cys, and Thr. Nevertheless, they have been used extensively in classical peptide synthesis in solution. They are also a reasonable choice to obtain peptide amides by reaction of the methyl ester with ammonia. Ethyl esters have a similar behavior to methyl esters but are more base-stable and, therefore, more prone to base-catalyzed side reactions.[185]

Carbamoylmethyl (Cam).[195,196] It is used for solution synthesis. It is removed by saponification with NaOH or Na_2CO_3 in DMF. It is removed selectively in the presence of Boc and Z. Nevertheless, it cannot be selectively removed in the presence of side-chain Bn-protected DTT in the presence of DIPEA in H_2O-AcCN to the exchange-labile Co(II) form. It has not been widely used since Asp.

4.3.3. Other Protecting Groups (Table 10)

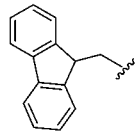
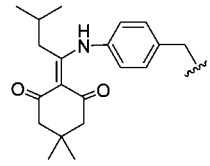
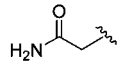
Allyl (Al).[164] It is removed using $Pd(PPh_3)_4$ (0.1 equiv) and $PhSiH_3$ (10 equiv) as scavenger in DCM within minutes or $Pd(PPh_3)_4$ and morpholine as nucleophile in THF−DMSO−0.5 M HCl (2:2:1), both on solid phase and in solution.[197] If removed on solid phase, washings with sodium *N*,*N*-diethyldithiocarbamate (0.02 M in DMF, 3 × 15 min) are carried out in order to remove Pd. Allyl *C*-terminal protection has been used for the synthesis of *C*-terminal modified peptides using the backbone linker (BAL) strategy,[39] and recently for the synthesis of peptide analogues where α-carboxyl protection is necessary both in solution and on solid phase, such as the synthesis of cyclic peptides via head-to-tail cyclization, among others.[198−202] In these cases, when the Al group from the carboxyl group and the Fmoc from the amino group need to be removed, it is preferable to first remove the Al and then the Fmoc. Removal of the Fmoc group first could increase the risk of allylation of the amino function during the removal of the Al.[201,203]

Benzyl (Bn). It is used mostly in solution synthesis. It is usually removed by catalytic hydrogenolysis. It can also be removed by saponification or hydrazinolysis to give the corresponding *C*-terminal hydrazide. Acidolytic removal is also possible, but harsh conditions are required. It is used in combination with the following N^{α}-protecting groups: Boc, Ddz, Bpoc, and Troc.[185]

Phenacyl (Pac).[204] It is used for synthesis in solution and removed by nucleophiles such as sodium thiophenoxyde or by treatment with Zn in AcOH.[204,205] It is degraded and only partially removed by catalytic hydrogenation. It is more electrophilic than the methyl ester, thereby making Pac-protected amino acids prone to racemization during coupling because of a reversible cyclization mechanism (Figure 7).

***p*-Nitrobenzyl (*p*NB).** It is highly resistant to acids and removed using a variety of reducing agents such as Na_2S,

Table 9. α-Carboxylic Acid-Protecting Groups Removed by Base

Name and Structure	Removal conditions	Stability to the removal of	Ref.
9-Fluorenylmethyl (Fm) 	15% DEA or 20% piperidine-DMF or DCM	Boc, Trt, Alloc	172, 190, 191, 192
4-(N-[1-(4,4-dimethyl-2,6-dioxocyclohexylidene)-3-methylbutyl]-amino)benzyl (Dmab) 	2% hydrazine-H_2O-DMF (1:1)	Boc, Fmoc, Trt,	193
Methyl (Me) and Ethyl (Et)	LiOH, NaOH or KOH	Boc, Z	185, 194
Carbamoylmethyl (Cam) 	NaOH or Na_2CO_3-DMF-H_2O	Boc, Fmoc [a] Z[b]	195, 196,

[a] Diethylamine removal. [b] Only catalytic hydrogenation removal.

$Na_2S_2O_4$, or $SnCl_2$ or by catalytic hydrogenation.[206−209] Solid-phase removal is performed by treatment with 8 M $SnCl_2$ in DMF containing 1.6 mM AcOH and 0.2% phenol for 5 h at 25 °C or three treatments of 30 min at 60 °C.[210] Washings with DMF, MeOH, and DMF, treatment with 8 M benzenesulfinic acid in DMF for 30 min at 25 °C, and further washings with DMF and MeOH are performed to eliminate the quinonimine methide formed during the removal.[211] Use of a less concentrated and more easy to handle 6 M $SnCl_2$ in DMF solution, substitution of AcOH by HCl in dioxane, and alternative washings (DMF, DMF/H_2O, THF/H_2O, DMF, and DCM, 3 × 30 s each) have been described in the case of Glu side-chain protection.[166] These conditions should be easily adapted to the removal of the *C*-terminal protecting group. Removal with TBAF in solution has also been proposed as an alternative to the reductive removal.[212]

2-Trimethylsilylethyl (TMSE).[213] It is removed with a quaternary ammonium fluoride such as TBAF or tetraethylammonium fluoride (TEAF) in DMF. It is stable to hydrogenolysis but unstable to anhydrous TFA. Nevertheless, Boc group can be removed selectively in its presence when HCl solutions in organic solvents are used.

(2-Phenyl-2-trimethylsiylyl)ethyl (PTMSE).[214,215] It is removed by treatment with TBAF·3 H_2O in DCM in almost neutral conditions within 3−5 min. It is stable to the hydrogenolytic cleavage of Z and Bn ester groups, base-induced removal of Fmoc groups, palladium(0)-catalyzed removal of Alloc, and even acidolytic cleavage of Boc groups if carried out under special conditions (*p*-TsOH or 1.2 M HCl in 2,2,2-trifluoroethanol (TFE). PTMSE esters are also stable under the conditions for amide bond formation in peptide synthesis or peptide condensation reactions, and therefore, they are considered valuable novel carboxy-protecting groups. However, no studies on how the use of PTMSE affects the formation of aspartimides have been performed to date.

2-(Trimethylsilyl)isopropyl (Tmsi).[216] It is used for peptide synthesis in solution. It is removed with TBAF (8 equiv) in THF in 1−1.5 h. It significantly reduces DKP formation in comparison with TMSE.

2,2,2-Trichloroethyl (Tce).[217] It is used mainly for solution synthesis. It is removed with Zn dust in AcOH in similar conditions as Troc and, therefore, can be removed in the presence of Z, Boc, Alloc, and Fmoc. Tce is stable even at pH 1, and therefore, Boc can be removed selectively in its presence. It is not completely stable to hydrogenolysis.

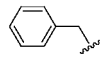
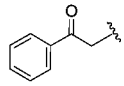
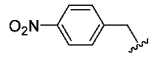
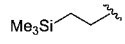
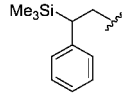
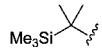
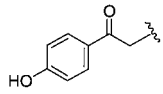
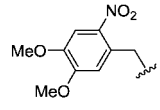
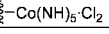
***p*-Hydroxyphenacyl (*p*HP).[218]** It is removed by photolysis (λ = 337 nm) and used as a new phototrigger. It is stable to Boc removal.

4,5-Dimethoxy-2-nitrobenzyl (Dmnb).[219] It is a photolabile protecting group analogous to the NVOC group. It has been used for the synthesis of misacylated tRNAs and recently for the synthesis of caged peptides.[220]

1,1-Dimethylallyl (Dma).[221] It is removed by treatment with $Pd(PPh_3)_4$ (10 mol %) in THF at room temperature, followed by dropwise addition of NMM (3 equiv) under nitrogen. $PhSiH_3$, potassium 2-ethyl hexanoate, or *p*-toluene sulfonic acid sodium salt can be used instead of NMM. It is orthogonal to the Fmoc group and can be removed in the presence of Bn- and ^{t}Bu-type groups, but it is not stable to their acidolytic removal.

Pentaamine Cobalt(III).[222] It was proposed as a *C*-terminal-protecting group for the synthesis of side chain to side chain bicyclic peptides. It is described as orthogonal to Fmoc and Boc and is removed in solution by mild reduction with then.

Table 10. Other α-Carboxylic Acid-Protecting Groups

Name and Structure	Removal conditions	Stability to the removal of	Ref.
Allyl (Al) 	$Pd(Ph_3)_4$ (0.1 eq.) and scavengers (usually $PhSiH_3$, 10 eq.)-DCM	Boc, Fmoc, *p*NZ,[a] Trt	39,164, 197,198, 199,200, 201,202, 203
Benzyl (Bn) 	1) HF 2) TFMSA 3) H_2 cat. 4) NaOH in aqueous organic solvents	Boc,[b] Fmoc, *p*NZ,[a] Trt, Alloc	185
Phenacyl (Pac) 	1) sodium thiophenoxyde 2) Zn in AcOH	Boc, Z,[a] Trt	204,205
p-Nitrobenzyl (pNB) 	1) $SnCl_2$ in DMF 2) $Na_2S{\cdot}9H_2O$-H_2O, 0-5ºC 3) $Na_2S_2O_4$, Na_2CO_3-H_2O, 40ºC 4) H_2 cat. 5) TBAF-THF, DMF or DMSO	Boc,Fmoc, Z,[a] Trt, Alloc	166,206 207,208, 209,210, 211,212
2-Trimethylsilylethyl (TMSE) 	TBAF or TEAF-DMF	Z[c]	213
(2-Phenyl-2-trimethylsilyl)ethyl (PTMSE) 	TBAF·3 H_2O-DCM	Fmoc, Z,[c] Alloc	214,215
2-(Trimethylsilyl)isopropyl (Tmsi) 	TBAF (8 eq.)-THF, 1-1.5 h	Z[c]	216
2,2,2-Trichloroethyl (Tce) 	Zn dust-AcOH	Boc, Fmoc, Trt	217
p-Hydroxyphenacyl (pHP) 	Photolysis (λ=337 nm)	Boc, Trt	218
4,5-Dimethoxy-2-nitrobenzyl (Dmnb) 	Photolysis (λ>320 nm)	Boc, Fmoc, Trt	219,220
1,1-Dimethylallyl (Dma) 	$Pd(PPh_3)_4$ and scavengers: NMM, $PhSiH_3$, potassium 2-ethyl hexanoate or *p*-Tos-OK-THF	Fmoc	221
Pentaamine cobalt (III) 	DTT, DIPEA-H_2O-AcCN	Boc, Fmoc, Trt	222

[a] Except catalytic hydrogenation removal. [b] Except repetitive removals. [c] Catalytic hydrogenation removal.

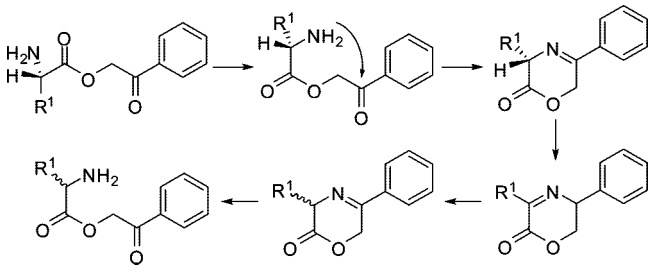


Figure 7. Racemization mechanism of Pac-protected amino acids.

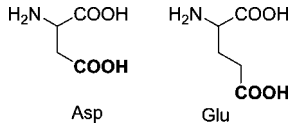


Figure 8. Aspartic (Asp) and glutamic (Glu) acids.

5. Aspartic (Asp) and Glutamic (Glu) Acids

5.1. General

The side-chain carboxylic groups of Asp and Glu (Figure 8) must be protected in order to prevent their activation during peptide synthesis, which would lead to undesired branched peptides.

Furthermore, in the case of Asp acid, the protecting groups used must also prevent or at least minimize the formation of aspartimide. Hydrolysis of the aspartimide during peptide synthesis renders two products: the α-peptide, which is the desired product, and the β-peptide, which is usally the major compound. Aminolysis of aspartimide by piperidine gives the corresponding α- and β-piperidides (Figure 9).

The same kind of intramolecular cyclization can also take place in the case of Glu, thereby leading to pyroglutamic formation.[223] However, in the case of Glu, the reaction is much less severe than with Asp.

Currently, the most used protecting groups are ^{t}Bu for the Fmoc/^{t}Bu strategy and, in the Boc/Bn strategy, the cyclohexyl (*c*Hx) group, which is replacing the classical Bn group because it is more effective at preventing the formation of aspartimide.

5.2. Introduction of the Protecting Groups

The protection of the side-chain carboxylic acid can be achieved using several methods. The simplest one is the acid-catalyzed esterification of the free amino acid, where protonation of the amino group makes the α-carboxylic acid less reactive, thereby allowing the selective protection of the side chain.[224,225]

Copper(II) and boron chelates used for the selective protection of the side chains of Lys and Orn are also applied for the selective protection of the side chains of Asp and Glu. After chelation and reaction with the appropiate protecting-group halide, the chelate is removed in the usual way.[181,182,142] Another alternative is the formation of an intramolecular anhydride between the two carboxylic acids, which leads to selective α-protection thanks to the major electrophilicity of the α-carboxylic acid. This allows the protection of the side chain with a distinct protecting group, followed by the removal of the α-carboxylic acid protection.[175,176]

5.3. Removal

5.3.1. Protecting Groups Removed by Acid (Table 11)

Benzyl (Bn).[185] It is the classical protecting group in Boc/Bn chemistry and is removed with HF or TFMSA. However, it is more prone to acid-catalyzed aspartimide formation than the cyclohexyl group. Other possible removal conditions are listed in the table.

Cyclohexyl (cHx). It is removed with HF or TFMSA.[226,227] It is widely used in the Boc/Bn solid-phase strategy. It is superior to the benzyl group at preventing acid-catalyzed aspartimide formation because of its major steric hindrance.[228] In addition, it is more resistant to acids than benzyl, thus making it more suitable for the synthesis of long peptides using the Boc/Bn strategy.

***tert*-Butyl (^{t}Bu).** It is removed with 90% TFA in DCM (solid phase and solution) or 4 M HCl in dioxane (solution). It is the most used protecting group in Fmoc/^{t}Bu chemistry, which is highly prone to aspartimide formation because of the reiterative use of piperidine. The ^{t}Bu group simply minimizes aspartimide formation because of its steric hindrance compared to other less-hindered protecting groups such as allyl. However, although the ^{t}Bu group is considered hindered in organic chemistry, it does not prevent aspartimide formation in those sequences prone to it.[229] See also the section on α-amino protection.

***β*-Menthyl (Men).**[230] It is removed with HF or TFMSA and is resistant to TFA. It leads to less base-catalyzed aspartimides than the cyclohexyl group but is not widely

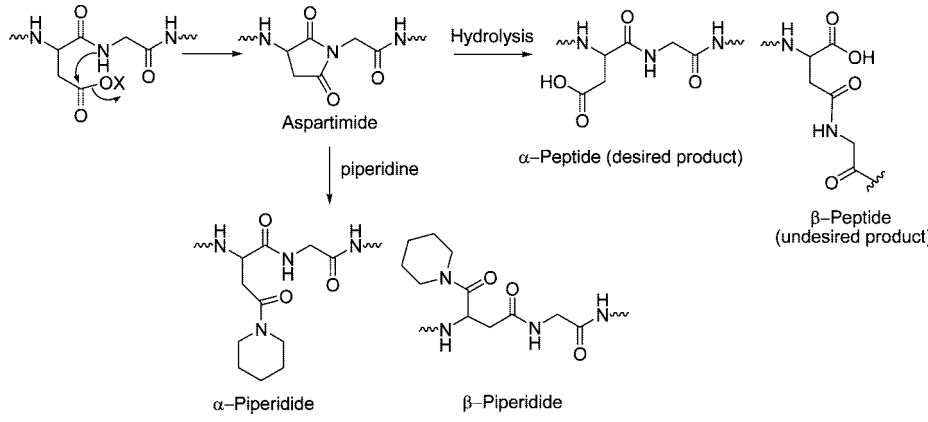
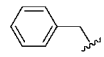
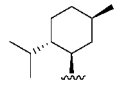
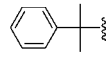
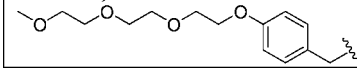


Figure 9. Aspartimide formation followed by piperidide formation upon piperidine treatment or hydrolysis rendering the α- and β-peptides.

Table 11. Asp and Glu-Protecting Groups Removed by Acid.

Name and Structure	Removal conditions	Stability to the removal of	Ref.
Benzyl (Bn) 	1) HF 2) TFMSA 3) H_2 cat. 4) NaOH in aqueous organic solvents	Boc, Fmoc, *p*NZ,[a] Trt, Alloc,	185
Cyclohexyl (cHx) 	1) HF 2) TFMSA	Boc, Fmoc, *p*NZ, Trt, Alloc	226, 227, 228
tert-Butyl (^{t}Bu) 	90% TFA-DCM (solid phase and solution) or 4 M $HCl_{(dioxane)}$ (solution)	Fmoc, Z,[b] Trt, Alloc, *p*NZ	229
β-Menthyl (Men) 	HF, TFMSA-TFA	Boc, Fmoc, Trt, Alloc, *p*NZ	230, 231
β -3-Methylpent-3-yl (Mpe) 	95% TFA-H_2O	Fmoc, Z, [b] Trt, Alloc	232
2-Phenylisopropyl (2-Ph^{i}Pr) 	1-2 % TFA-DCM	Fmoc, Alloc	188, 233, 234, 235
4-(3,6,9-Trioxadecyl)oxybenzyl (TEGBz or TEGBn) 	TFA-DCM	Fmoc, Trt	236

[a] Except catalytic hydrogenation removal. [b] Only catalytic hydrogenation removal.

used. Sometimes diphenyl sulfide should be added as a scavenger to facilitate Men removal.[231]

***β*-3-Methylpent-3-yl (Mpe).[232]** It is removed with 95% TFA and is more sterically hindered than the ^{t}Bu group and, therefore, less prone to aspartimide formation.

2-Phenylisopropyl (2-Ph^{i}Pr). It is removed with 1−2% TFA.[233,188] It is used in the Fmoc/^{t}Bu strategy mostly for the protection of Glu but also of Asp.[234] It can be removed in the presence of ^{t}Bu-type protecting groups, and therefore, it is useful for the preparation of cyclic peptides.[235]

4-(3,6,9-Trioxadecyl)oxybenzyl (TEGBz or TEGBn).[236] It is a recently developed protecting group that is removed with TFA−DCM. It has been used for the solid-phase synthesis of "difficult" peptide sequences (those very prone to aggregate) because it minimizes chain aggregation during the synthesis.

5.3.2. Protecting Groups Removed by Base (Table 12)

9-Fluorenylmethyl (Fm).[142,237,238] It is removed with secondary amines such as diethylamine or piperidine in DMF. It is stable to HBr in AcOH and TFA/thioanisole, nonstable to catalytic hydrogenation, and not completely stable to HF even at 0 °C. It is used for the Boc/Bn strategy when orthogonal protection of the side chains is required.

4-(*N*-[1-(4,4-Dimethyl-2,6-dioxocyclohexylidene)-3-methylbutyl]amino)benzyl (Dmab).[193,239] It is removed with 2% hydrazine within minutes in DMF−H_2O. It is stable to 20% piperidine in DMF and TFA. Nevertheless, in some cases, it can lead to pyroglutamyl-terminated peptides.[240]

5.3.3. Other Protecting Groups (Table 13)

Benzyl (Bn). See the section on protecting groups removed by acid.

Allyl (Al).[164,165,241] It is removed with palladium and stable to TFA and bases. See also the section on α-carboxylic acid protection.

***p*-Nitrobenzyl (*p*NB).[242]** It promotes aspartimide formation when used to protect Asp. See also the section on α-carboxylic acid protection.

2-(Trimethylsilyl)ethyl (TMSE).[213,243] It is removed with fluorides, is unstable to acids and bases, and is stable to hydrogenolysis. It is used for the protection of Asp acid for cyclization on a Rink amide resin.[244]

(2-Phenyl-2-trimethylsiylyl)ethyl (PTMSE). See the section on α-carboxylic acid protection.

4,5-Dimethoxy-2-nitrobenzyl (Dmnb). See the section on α-carboxylic acid protection.

Table 12. Asp and Glu-Protecting Groups Removed by Base

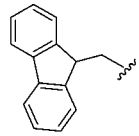
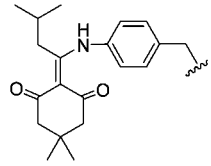
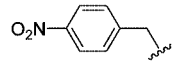
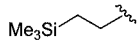
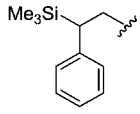
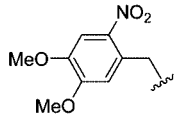
Name and Structure	Removal conditions	Stability to the removal of	Ref.
9-Flurorenylmethyl (Fm) 	Secondary amines: 15% DEA or 20% piperidine-DMF or DCM	Boc, Trt, Alloc	142, 237, 238
4-(N-[1-(4,4-dimethyl-2,6-dioxocyclohexylidene)-3-methylbutyl]-amino)benzyl *(Dmab)* 	2% hydrazine-DMF-H_2O	Boc, Fmoc, Trt, Alloc	193, 239, 240

Table 13. Other Asp and Glu-Protecting Groups

Name and Structure	Removal conditions	Stability to the removal of	Ref.
Allyl (Al) 	$Pd(Ph_3)_4$ (0.1 eq.) and scavengers (usually $PhSiH_3$, 10 eq.) in DCM	Boc, Fmoc, *p*NZ, [a] Trt	164, 165, 241
p-Nitrobenzyl (pNB) 	1) $SnCl_2$ in DMF 2) $Na_2S{\cdot}9H_2O$ in H_2O, 0-5°C 3) $Na_2S_2O_4$, Na_2CO_3 in H_2O, 40°C 4) H_2 cat. 5) TBAF-THF, DMF or DMSO	Boc,Fmoc, Z,[a] Trt, Alloc	242
Trimethylsilylethyl (TMSE) 	TBAF or TEAF-DMF	Z [b]	213, 243, 244
(2-Phenyl-2-trimethylsilyl)ethyl (PTMSE) 	TBAF·3 H_2O-DCM	Fmoc, Z, [b] Alloc	214, 215
4,5-Dimethoxy-2-nitrobenzyloxycarbonyl (Dmnb) 	Photolysis (λ>320 nm)	Boc, Trt	219, 220

[a] Except catalytic hydrogenation removal. [b] Catalytic hydrogenation removal.

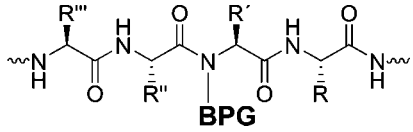


Figure 10. Partially backbone-protected peptide; BPG = backbone-protecting group.

6. Amide Backbone

6.1. General

The NH backbone is usually unprotected in peptide synthesis. However, at least three undesired interactions involving the NH backbone have been described.

First of all, peptide chains can aggregate during the synthesis as a result of intra- and intermolecular interactions, thereby significantly reducing coupling and deprotection yields.[245−247] Backbone protection (Figure 10) minimizes these aggregation phenomena by preventing the formation of hydrogen bonds and also because of steric hindrance. Thus, SPS of long peptidic sequences prone to aggregation is improved by protecting some amides of the peptide.[248−251]

Second, nucleophilic attack of the amide NH of the amino acid before an Asp residue (usually Gly, Ser, or Thr)[70,227,63,252−255] to the β-carboxyl group of Asp renders aspartimide and the subsequent formation of β-peptide and other side products. (See the section on Asp and Glu side-chain protection.) Aspartimide formation is more severe in the Fmoc/^{t}Bu strategy and with the Asp-Gly sequence, but it can occur in many other cases. Finally, although less frequent, internal DKP formation involving the NH and the activated carboxylic acid of the previous amino acid has recently been described during fragment coupling (Figure 11)[122]

The most used backbone protectors for the Fmoc/^{t}Bu strategy are pseudoprolines (Figure 12),[256,257,251] 2-hydroxy-4-methoxybenzyl (Hmb),[258] 2,4-dimethoxybenzyl (Dmb), and more recently 3,4-ethylenedioxy-2-thenyl ($EDOT_n$) and 1-methyl-3-indolylmethyl (MIM)[259] The pseudoproline concept is valid only for β-hydroxy or thio amino acids such as Ser/Thr or Cys. Although the rest of protecting groups can be used for all amino acids, practically they are only used for Gly because of the difficulty of elongation of the peptide chain due to steric hindrance [260]

6.2. Introduction of the Protecting Groups

Because of the steric hindrance of the protected amino acid, it is incorporated usually through the corresponding derivatives. Thus, pseudoproline dipeptides are prepared by reaction of Fmoc-AA-Ser or Fmoc-AA-Thr with 2,2-dimethoxypropane.[261] Most of the other backbone protectors are introduced by reductive amination of the aldehyde of the protecting group with the amine of the corresponding amino acid, followed by either α-amino protection or dipeptide formation.[262,258,259]

6.3. Removal

6.3.1. Protecting Groups Removed by Acid (Table 14)

Pseudoprolines (ΨPro). The most used are dimethyloxazolidines ($\Psi^{Me,Me}$Pro) because of their major acid lability (removed by TFA within minutes)[261] Pseudoproline derivatives have been extensively applied to the synthesis of difficult peptides.[257,263] However, they are limited to Ser and Thr. Dimethylthiazolidines (Cys pseudoprolines) have also been described, but they are not so widely used because of their major acid stability (removed with TFA within hours).

2-Hydroxy-4-methoxybenzyl (Hmb).[258] It is used mainly as Fmoc-(FmocHmb)AA1-OH[264] or as Fmoc-AA2-(Hmb)-AA1-OH but also as Fmoc(Hmb)AA1-OH.[265] It is removed with TFA. The main advantage of the Hmb group compared with other backbone protectors such as Dmb is that the coupling on Hmb-amino acids is easier. Thus, Hmb is not restricted to Gly, and derivatives of more hindered amino acids can be used. However, the presence of a free hydroxyl group can be a problem in depsipeptide synthesis or in postsynthetic phosphorylations.

2,4-Dimethoxybenzyl (Dmb).[266] It is removed with high concentrations of TFA. Its major inconvenience is its bulkiness, which limits its use for nonsterically hindered

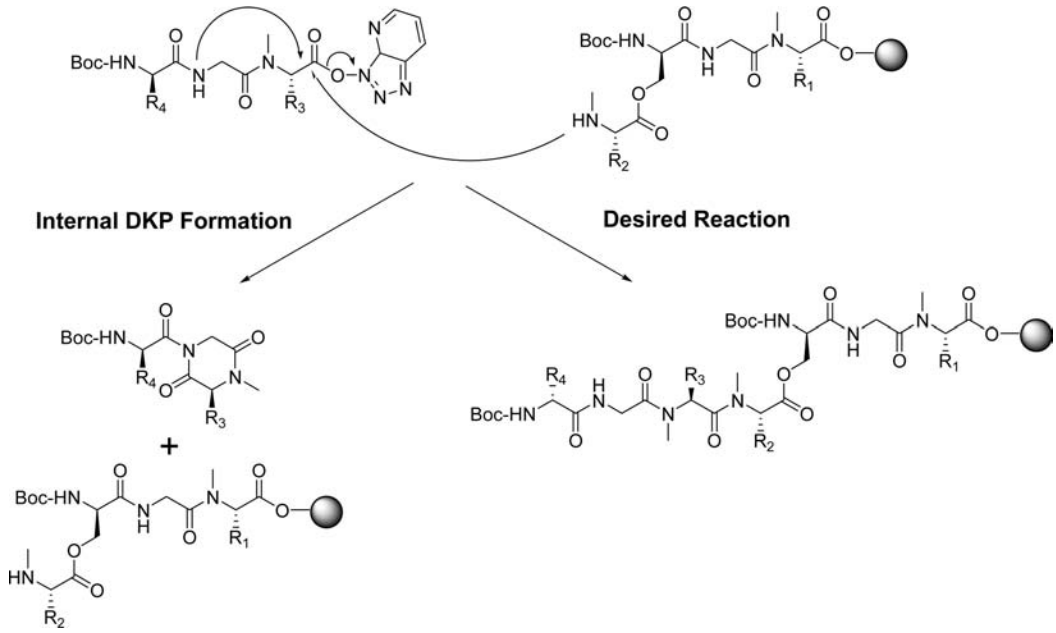


Figure 11. Internal DKP formation. Adapted with permission from ref 122. Copyright 2007 American Chemical Society.

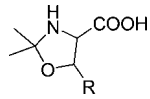


Figure 12. Pseudoproline of Ser (R = H) and Thr (R = CH_3).

amino acids (mainly Gly),[255] or for direct coupling of Dmb-protected dipeptides (Fmoc-AA′-(Dmb)AA-OH). [267]

2,4,6-Trimethoxybenzyl (Tmob).[268] It is removed with TFA and has been used for the Fmoc/^{t}Bu SPS of highly hydrophobic peptides.[269] Although it is not as widely used as Dmb, coupling on 2,4,6-trimethoxybenzylamines of amino acids is described to be faster than in the case of the less-hindered 2,4-dimethoxybenzylamines.[258]

1-Methyl-3-indolylmethyl (MIM) and 3,4-Ethylenedioxy-2-thenyl (EDOT$_n$).[259] These are recently developed backbone protectors for the Fmoc/^{t}Bu strategy. They are completely removed with TFA−DCM−H_2O (95:2.5:2.5) in 1 h. Both are more acid-labile than the 2,4-dimethoxybenzyl group, and EDOT$_n$ is less sterically hindered, thus couplings on EDOT$_n$ amino acids are faster.

6.3.2. Other Protecting Groups (Table 15)

4-Methoxy-2-nitrobenzyl.[270] It is removed by photolysis at λ = 360 nm for more than 2 h using Cys (200 mmol/(1 mmol of 4-methoxy-2-nitrobenzyl)) as scavenger. This is a backbone protector, fully compatible with Boc chemistry, thereby allowing the obtention of backbone-protected peptides after HF cleavage.

(6-Hydroxy-3-oxido-1,3-benz[d]oxathiol-5-yl)methyl.[271,272] and 2-hydroxy-4-methoxy-5-(methylsulfinyl)benzyl.[273] These are safety-catch backbone protectors that become unstable to TFA after reduction of the sulfoxide to sulfide. (6-Hydroxy-3-oxido-1,3-benzoxathiol-5-yl)methyl is removed with 20 equiv each of NH_4I and $(CH_3)_2S$ in TFA at 0 °C

Table 14. Amide Backbone-Protecting Groups Removed by Acid

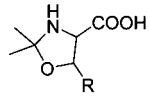
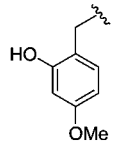
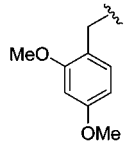
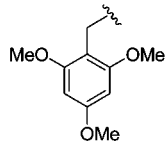
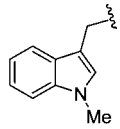
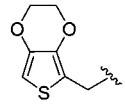
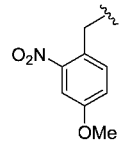
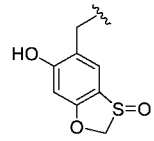
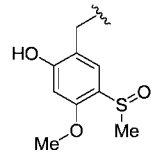
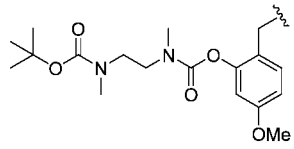
Name and Structure	Removal conditions	Stability to the removal of	Ref.
Pseudoprolines (oxazolidines) ***Pseudoprolines***  R= H (Ser) or Me (Thr)	95% TFA and scavengers	Fmoc, Alloc	257, 261, 263
2-Hydroxy-4-methoxybenzyl (Hmb) 	95% TFA and scavengers	Fmoc, Alloc	258, 264, 265
2,4-Dimethoxybenzyl (Dmb) 	95% TFA and scavengers	Fmoc, Alloc	255, 266, 267
2,4,6-Trimethoxybenzyl (Tmob) 	95% TFA and scavengers	Fmoc, Alloc	258, 268, 269
1-Methyl-3-indolylmethyl (MIM) 	TFA-DCM-H_2O (95:2.5:2.5)	Fmoc, Alloc	259
3,4-Ethylenedioxy-2-thenyl (EDOT$_n$) 	TFA-DCM-H_2O (95:2.5:2.5)	Fmoc, Alloc	259

Table 15. Other Amide Backbone-Protecting Groups

Name and Structure	Removal conditions	Stability to the removal of	Ref.
4-Methoxy-2-nitrobenzyl 	Photolysis (λ=360 nm) and Cys as scavenger	Boc, Z, [a] Trt, Alloc	270
(6-Hydroxy-3-oxido-1,3-benz[d]oxathiol-5-yl)methyl 	NH_4I (20 eq.) and $(CH_3)_2S$ (20 eq.)-TFA, 2 h, 0°C.	Boc, Fmoc,Trt	271, 272
2-Hydroxy-4-methoxy-5-(methylsulfinyl)benzyl 	$SiCl_4$-TFA-anisole-ethanedithiol, (5:90:2.5:2.5), 2 h, rt	Boc, Fmoc,Trt	273
N-Boc-N'-methyl[2-(methylamino)ethyl]carbamoyl-Hmb (Boc-Nmec-Hmb) 	i) 25-50% TFA-DCM ii) *N*-methylmorpholine (10 eq) in DMF/H2O (3:7), 4 -8 h iii) 95% TFA and scavengers	Fmoc, Trt	274

[a] Except catalytic hydrogenation removal.

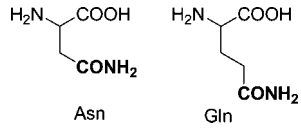


Figure 13. Asparagine (Asn) and Glutamine (Gln).

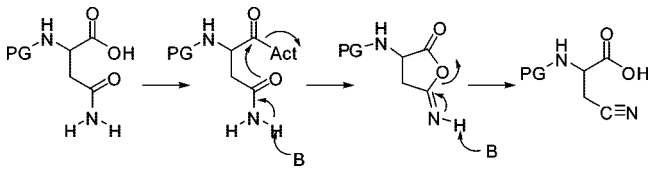


Figure 14. Dehydratation of Asn.

over 2 h, whereas 2-hydroxybenzyl-4-methoxy-5-(methylsulfinyl) is removed with $SiCl_4$−TFA−anisole−ethanedithiol, (5:90:2.5:2.5), for 2 h at room temperature. Acylation as well as acyl migration is faster in the case of the latter.

Boc-*N*-methyl-*N*-[2-(methylamino)ethyl]carbamoyl-Hmb (Boc-Nmec-Hmb).[274] It is a recently developed protecting group. It has been used for solid-phase synthesis. After the removal of the Boc group with TFA during the cleavage of the peptide from the resin, the Nmec moiety is removed via an intramolecular cyclization in basic conditions (*N*-methylmorpholine (10 equiv) in DMF/H2O (3:7), 4−8 h), leading to the Hmb-protected peptide. Then, Hmb is removed with 95% TFA and scavengers. The main advantage of the Boc-Nmec-Hmb group is that, after Boc removal, a cationic peptide is obtained that increases the solubility of insoluble peptides, making their purification easier.

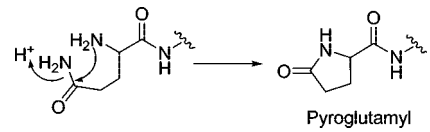


Figure 15. Pyroglutamyl formation.

7. Asparagine (Asn) and Glutamine (Gln)

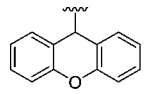
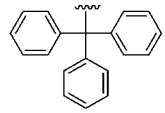
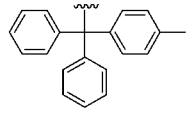
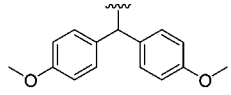
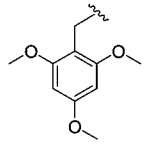
7.1. General

Asn and Gln (Figure 13) are often used without side-chain protection.

Nevertheless, unprotected derivatives show poor solubility and, therefore, slow coupling rates. In addition, their free primary amides can undergo two main side reactions:

(1) Dehydratation during the coupling (Figure 14), which is a base-catalyzed side reaction and, therefore, more favored in those coupling protocols that involve the use of base. It can be minimized using the corresponding N^{α}-protected pentafluorophenyl esters or

Table 16. Asn- and Gln-Protecting Groups Removed by Acid

Name and Structure	Removal conditions	Stability to the removal of	Ref.
9-Xanthenyl (Xan) 	90% TFA-scavengers	Fmoc, Trt, Alloc	278, 281, 282
Trityl (Trt) 	TFA-H_2O-EDT (90:5:5)	Fmoc, Trt, Alloc	279, 280
4-Methyltrityl (Mtt) 	95% TFA	Fmoc, Trt, Alloc	279, 283
Cyclopropyldimethylcarbinyl (Cpd) 	TFA-thioanisole-EDT-anisole (90:5:3:2)	Fmoc, Alloc	284, 285
4,4'-Dimethoxybenzhydryl (Mbh) 	1 M TMSBr-thioanisole-EDT-*m*-cresol in TFA (2 h at 0°C)	Fmoc, Alloc	275, 286, 287
2,4,6-Trimethoxybenzyl (Tmob) 	95% TFA-DCM and scavengers	Fmoc, Alloc	280, 288, 289

carbodiimide-mediated couplings in the presence of HOBt.[275,276] Dehydratation is more important in the Fmoc/^{t}Bu strategy than in the Boc/Bn one; in the latter, the use of HF apparently reverts the reaction, whereas in the former, TFA is not acidic enough to revert to the amide.[276]

(2) Pyroglutamyl (Figure 15) formation is a weak acid-catalyzed side reaction that occurs on *N*-terminal Gln that leads to truncated peptidic chains. Being an acid-catalyzed reaction, it has more importance in the Boc/Bn strategy and can be minimized by reducing exposure to weak acids.[277]

Adequate protection of Asn and Gln side chains prevents both side reactions. As for dehydratation, it is not necessary for the protecting group of choice to be stable during the whole peptide synthesis, but only during the coupling step. Furthermore, protection of Asn and Gln side chains also increases coupling yields by confering more solubility to the corresponding Asn and Gln derivatives and probably reducing the formation of hydrogen bonds that stabilize secondary structures.

Removal of the protecting groups is usually easier in Gln than in Asn, being particularly difficult in *N*-terminal Asn because of the proximity of the free and therefore protonated α-amino group.[278−280]

Currently, the most used protecting groups are Xan (9-xanthenyl) and Trt, which are compatible with both Boc/Bn and Fmoc/^{t}Bu strategies. In the case of the former, the Xan group protects Asn and Gln side chains only during the coupling and is removed during TFA treatments for Boc removal.

7.2. Introduction of the Protecting Groups

Protection is usually performed via acid-catalyzed reaction of the corresponding alcohol with Z-Gln or Z-Asn, followed by catalytic hydrogenolysis to eliminate the Z group and Fmoc or Boc N^{α} protection.[280,281] In the case of 9-xanthenyl, the direct protection of the Fmoc-Asn and Fmoc-Gln has also been described.[282]

7.3. Removal

7.3.1. Protecting Groups Removed by Acid (Table 16)

9-Xanthenyl (Xan).[281] It is removed by 90% TFA and scavengers. In contrast to Trt, no extra reaction time is required when the α-amino of Asn is free.[278] Xan is used in both the Boc/Bn and Fmoc/^{t}Bu strategies.[281,282] In the case of the Boc strategy, Xan is eliminated during TFA treatments to remove the Boc group; however, Asn or Gln residues can undergo dehydratation only during the coupling, and thus, Xan elimination after it is a minor problem.[282]

Trityl (Trt).[279,280] It is removed with TFA−H_2O−EDT (90:5:5) and used in both the Boc/Bn and Fmoc/^{t}Bu strategies. The time required for removal increases from 10

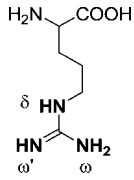


Figure 16. Arginine (Arg) .

min to more than 4 h when the α-amino of Asn is free. Scavengers must be used to prevent Trp alkylation. It is stable to bases and catalytic hydrogenolysis.

4-Methyltrityl (Mtt).[283,279] It is a more acid-labile alternative to the Trt group (95% TFA, 20 min) and is particularly useful when the α-amino of Asn is free.

Cyclopropyldimethylcarbinyl (Cpd).[284,285] It is removed with TFA−thioanisole−EDT−anisole (90:5:3:2), being another more acid-labile alternative to Trt, especially when the α-amino of Asn is free. It is more soluble and coupling rates are better than with the Trt group.

4,4′-Dimethoxybenzhydryl (Mbh).[286,287] It is used mainly in the Boc/Bn strategy but also in the Fmoc/^{t}Bu one. Its removal using TFA is slow and requires scavengers to prevent alkylation of Trp.[275]

2,4,6-Trimethoxybenzyl (Tmob).[288] It is removed with 95% TFA and scavengers. It is more acid-labile, more soluble, and gives less side reactions during coupling than Mbh-protected derivatives. However, it is not currently widely used because it can cause alkylation of Trp and is reported to give worse results than the Trt group.[289,280]

8. Arginine (Arg)

8.1. General

Protection of the guanidino group of Arg (Figure 16) is required to prevent deguanidination, which renders Orn (Figure 17)[290] and δ-lactam formation (Figure 18) as a result of the nucleophilicity of the guanidino group. Arg side-chain protection remains unsolved in peptide synthesis because of the difficulty to remove the protecting groups.

Since the guanidino group is basic (pK_a = 12.5), it remains protonated in most of the conditions used for peptide synthesis.[291,292] To prevent deprotonation in Fmoc/^{t}Bu SPS, washings with 0.25 M HOBt are carried out between Fmoc removal and the next coupling.[293] However, if deprotonation takes place, deguanidination occurs after acylation of the neutral guanidino group. This drawback stimulated research into protecting groups for Arg.

Arg derivatives tend to be worse acylating reagents compared with other amino acid derivatives, mainly because of the formation of the δ-lactam from the activated species (Figure 18). In a solid-phase mode, the presence of the δ-lactam does not translate into an impurity in the crude reaction, because it is not reactive but it is translated in a less active species to be coupled.

In principle, protection of all the nitrogens of the guanidino group is required to fully mask its nucleophilicity. However, diprotection and monoprotection are easier to achieve and to minimize side reactions when bulky and electron-withdrawing protecting groups are used.

The most used protecting strategy is sulfonyl protection of the ω-amino function. For the Boc/Bn strategy, the most used group is Tos, while for the Fmoc/^{t}Bu strategy, the most popular protecting groups are Pbf (pentamethyl-2,3-dihydrobenzofuran-5-sulfonyl) and Pmc (2,2,5,7,8-pentamethylchroman-6-sulfonyl). However, both, but particularly Pmc, are too acid-stable and their removal in peptides with multiple Args is especially problematic.

8.2. Introduction of the Protecting Groups

It depends on the nature of the protecting group. In the case of sulfonyl-protecting groups, which are the most used ones, they are usually introduced by reaction of the corresponding sulfonyl chloride with Z-Arg-OH in H_2O−acetone using NaOH as a base. To obtain the corresponding Fmoc/Boc derivative, the Z group is removed by catalytic hydrogenolysis and the Fmoc/Boc group is incorporated under regular conditions.[294]

8.3. Removal

8.3.1. Protecting Groups Removed by Acid (Table 17)

8.3.1.1. Arylsulfonyl ω-Protection. Although this kind of protection does not fully prevent δ-lactam formation, this process can be minimized by using carbodiimides in the presence of HOBt derivatives to decrease the activity of the active *O*-acylisourea.[185]

Tosyl (Tos). It is removed with HF, TFMSA−TFA−thioanisole, or Na/NH_3.[295] It is the most used protecting group in the Boc/Bn solid-phase strategy.[296]

2,2,5,7,8-Pentamethylchroman-6-sulfonyl (Pmc).[294] It is widely used in the Fmoc/^{t}Bu solid-phase strategy. It is removed by TFA scavengers. Currently, it is being replaced by the Pbf group.

2,2,4,6,7-Pentamethyl-2,3-dihydrobenzofuran-5-sulfonyl (Pbf).[297] It is removed by TFA scavengers and is more acid-labile than the Pmc group. Currently, it is the best Arg-protecting group for the Fmoc/^{t}Bu solid-phase strategy, although it is still too acid- stable in peptides with multiple Arg-containing peptides where long reaction times are required.

Mesityl-2-sulfonyl (Mts)[298,299] It is removed with TFMSA−TFA−thioanisole. It is used in the Boc/Bn solid-phase strategy and is more acid-labile than the tosyl group.

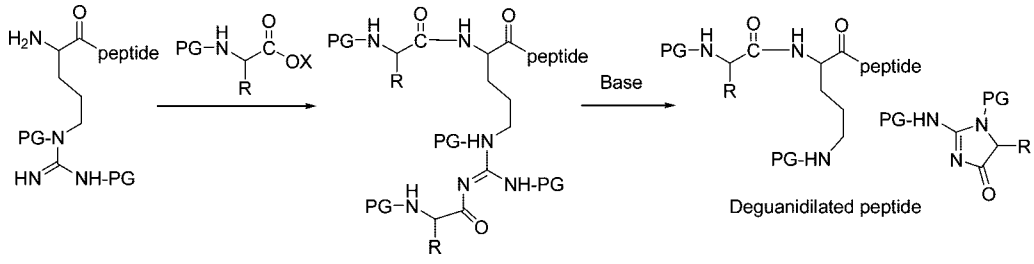


Figure 17. Acylation of the side chain of Arg during amino acid coupling, followed by base-catalyzed deguanidination. Adapted with permission from ref 290. Copyright 1984 Elsevier.

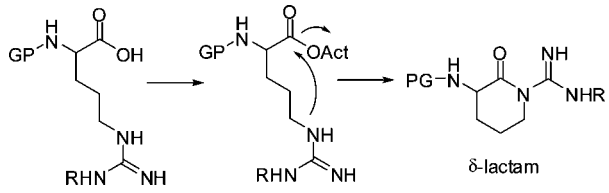


Figure 18. Mechanism of δ-lactam formation; R = H or protecting group.

4-Methoxy-2,3,6-trimethylphenylsulfonyl (Mtr).[300] It is removed by TFA−thioanisole. Although it is still used, it has been mostly replaced by the more acid-labile Pmc or Pbf in Fmoc/^{t}Bu chemistry.

1,2-Dimethylindole-3-sulfonyl (MIS).[301] It is a recently developed protecting group, which is much more TFA-labile than Pbf. It is completely removed with 50% TFA in 30 min, even in multiple Arg-containing peptides.

8.3.1.2. Other Kinds of Arg Protection. *ω,ω′-bis-tert-Butyloxycarbonyl (bis-Boc).*[302] It is removed with 90−95% TFA in the presence of scavengers and prevents deguanidination but does not completely prevent δ-lactam formation.[303,304] The coupling rates of bis-Boc-protected Arg are low.

ω-5-Dibenzosuberenyl (Suben), 5-Dibenzosuberyl (Sub), and 2-Methoxy-5-dibenzosuberyl (MeSub).[305] They are the most acid-labile derivatives (removed with 25−50% TFA) and are reported to minimize δ-lactam formation and deguanidination because of their steric hindrance. Although they look very promising, they have not been widely used.

ω-Nitro (NO_2).[306] It prevents δ-lactam formation and deguanidination in most cases. It can be removed with HF (SPS) or catalytic hydrogenolysis. In both cases, long reaction times are required, which is an inconvenience in the case of sensitive peptides. For instance, in the case of hydrogenolysis, partial hydrogenation of Trp or even Phe can occur.[307] Because of the clean removal of the nitro group by hydrogenolysis and its low cost, nitro protection is still used for large-scale solution synthesis of peptides [308,309] and even for SPS, where the nitro group is removed by hydrogenolysis after the cleavage from the resin.[310]

8.3.2. Protecting Groups Removed by Base (Table 18)

Trifluoroacetyl (tfa). It has been applied recently for the protection of guanidines used in solution Boc peptide synthesis and Fmoc/^{t}Bu SPPS. However, although there are references of tfa-protected Arg derivatives,[311−313] to date it has not been implemented for Arg protection in peptide synthesis.

8.3.3. Other Protecting Groups (Table 19)

Nitro (NO_2). See the section on protecting groups removed by acid.

***ω,ω′*-bis-Benzyloxycarbonyl (bis-Z).**[314] Its removal by catalytic hydrogenation requires long reaction times. It is used mostly in Boc/Bn chemistry but also in the Fmoc/^{t}Bu strategy.

***ω,ω′*-bis- Allyloxycarbonyl (Alloc).**[165] It is removed with $Pd(PPh_3)_4$ and scavengers (dimethylbarbituric acid)[315] and is compatible with the Boc/Bn solid-phase strategy. The base treatment required to remove the Fmoc group also eliminates one of the Alloc groups.

9. Cysteine (Cys)

9.1. General

Protection of the side chain of Cys (Figure 19) is mandatory in peptide synthesis because the nucleophilic thiol can otherwise be acylated, alkylated, or oxidized to disulfide by air.

Nevertheless, even protected Cys can undergo several side reactions. The most relevant are listed here:

• Oxidation and alkylation of the thioether. Although less critical than in the case of Met, it can also occur.[316−318] Oxidation of the Cys residues during global deprotection can be minimized using 10% of H_2O as scavenger.[122]

• β-Elimination (Figure 20) occurs when protected Cys is exposed to strong bases, such as sodium in liquid ammonia (required to remove the Benzyl group), alkaline conditions, or hydrazylnolysis, or exposed to strong acids such as HF. This side reaction is particularly critical in the case of *C*-terminal Cys, which in the Fmoc/^{t}Bu strategy undergoes β-elimination followed by piperidine addition to give piperidylalanine residue. The extent of β-elimination also depends strongly on the protecting group used, with S^{t}Bu being the worst case followed by Acm and Trt.[319,320] The Bn group can also produce β-elimination.

• Reaction with carbocations resulting from the elimination of protecting groups: after its deprotection, Cys can react with the cations generated in acidic conditions. For instance, *S-tert*-butylated Cys has been observed after the removal of the Boc group or after global deprotection in a Fmoc/^{t}Bu strategy.[321]

• Reattachment to the resin: resin-bound carbocations generated in the acidolytic cleavage from resins can react with both protected and unprotected Cys, thus causing reattachment of the peptide to the resin.[322]

- Transfer of Acm (acetamidomethyl) group to Ser, Thr, Gln, and Tyr during Acm removal.[323−325]

- Formation of thiazolidines of *N*-terminal Cys (Figure 21) can take place if His- protecting groups such as Bom (benzyloxymethyl) or Bum (*tert*-butyloxymethyl), which generate formaldehyde when removed, are present. It can be minimized using Cys as scavenger.[326,327]

- Racemization: Cys is highly prone to racemize during the anchoring to the solid support or during the couplings.[328,329] The extent of the racemization also depends on the *S*-protecting groups (S^{t}Bu > Trt > Acm > MeBn > ^{t}Bu)[330−334] and coupling methods used (favored if preactivation in the presence of base is performed and in the coupling methods involving the use of base). Epimerization of the Cys linked to a hydroxyl resin can even take place during the synthesis as a result of the repetitive base treatments to remove the Fmoc group, with 2-chlorotrityl resin being the least prone to this process.[330,335]

The most used protecting groups for the Fmoc/^{t}Bu strategy are the Acm or Trt groups, when the desired product is the disulfide, and the Trt group, when the desired product is the free thiol. For the Boc/Bn strategy, the most used are Bn and Meb (*p*-methylbenzyl) to obtain the free thiol and Acm to obtain disulfides.

9.2. Introduction of the Protecting Groups

The Cys thiol shows high nucleophilicity; therefore, Cys thiol protection is usually carried out using fully unprotected Cys as starting material. The *S*-protecting agents used can

Table 17. Arg-Protecting Groups Removed by Acid

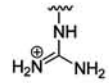
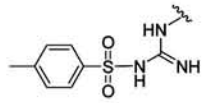
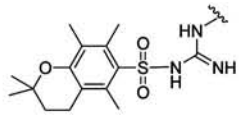
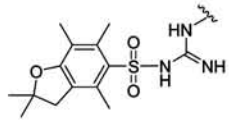
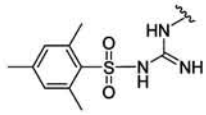
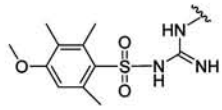
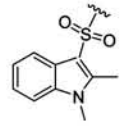
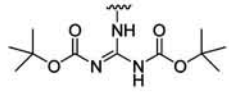
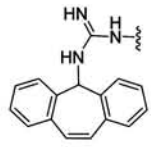
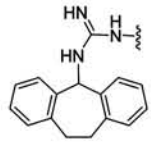
Name and Structure	Removal conditions	Stability to the removal of	Ref.
Protonation 	-	-	293
p-Toluenesulfonyl (Tos) 	1) HF 2) TFMSA-TFA-thioanisole 3) Na/NH_3	Boc, Fmoc, Trt, Alloc	295, 296
2,2,5,7,8-Pentamethylchroman-6-sulfonyl (Pmc) 	90% TFA-scavengers (H_2O and TIS) several hours.	Fmoc, Trt, Alloc	294
2,2,4,6,7-Pentamethyl-2,3-dihydrobenzofuran-5-sulfonyl (Pbf) 	90 TFA-scavengers (H_2O and TIS) 1 h (longer times in multiple arginine containing peptides)	Fmoc, Trt, Alloc	297
Mesityl-2-sulfonyl (Mts) 	TFMSA-TFA-thioanisole	Boc, Fmoc, Trt, Alloc	298, 299
4-Methoxy-2,3,6-trimethylphenylsulfonyl (Mtr) 	95% TFA-thioanisole	Fmoc, Trt, Alloc	300
1,2-Dimethylindole-3-sulfonyl (MIS) 	50% TFA and scavengers, 30 min	Fmoc, Alloc	301
ω,ω'-bis-tert-Butyloxycarbonyl (bis-Boc) 	90- 95% TFA and scavengers	Fmoc, Alloc	302, 303, 304
5-Dibenzosuberenyl (Suben) 	25-50% TFA	Fmoc, Alloc	305
5-Dibenzosuberyl (Sub) 	25-50% TFA	Fmoc, Alloc	305

Table 17. (Continued)

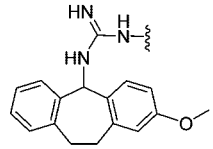
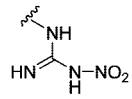
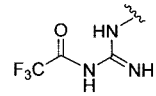
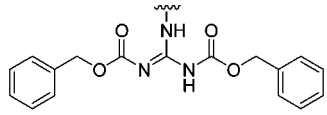
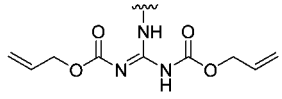
Name and Structure	Removal conditions	Stability to the removal of	Ref.
2-Methoxy-5-dibenzosuberyl (MeSub) 	25-50% TFA	Fmoc, Alloc	305
Nitro (NO_2) 	HF (solid phase) H_2 cat. (solution)	Boc, Fmoc, Alloc	306, 307, 308, 309, 310

Table 18. Arg-Protecting Groups Removed by Base

Name and Structure	Removal conditions	Stability to the removal of	Ref.
Trifluoroacetyl (tfa) 	1) K_2CO_3-MeOH-H_2O (solution) 2) K_2CO_3-MeOH-DMF-H_2O (solid phase)	Boc, Fmoc, Z,[a] Trt, Alloc	311, 312, 313

[a] Catalytic hydrogenation removal.

Table 19. Other Arg-Protecting Groups

Name and Structure	Removal conditions	Stability to the removal of	Ref.
ω,ω'-bis-Benzyloxycarbonyl (Z) 	H_2 cat. (long time)	Boc, Fmoc, Trt	314
ω,ω'-bis- Allyloxycarbonyl (Alloc) 	$Pd(PPh_3)_4$, barbituric acid	Boc, Fmoc, Z,[a] Trt	165, 315

[a] Except catalytic hydrogenation removal.

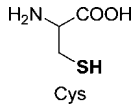


Figure 19. Cysteine (Cys).

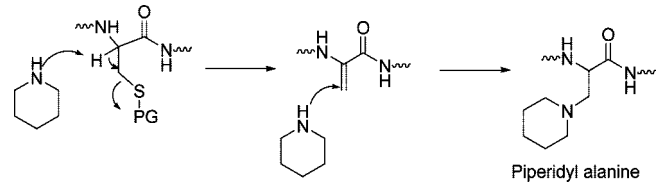


Figure 20. Base-catalyzed β-elimination of protected Cys followed by piperidine addition leading to piperidyl alanine.

be alkyl halides or tosylates, under acidic or basic conditions, or alcohols, which are dehydrated under acidic conditions. Benzyl-type protection can also be performed via reduction of the thiazolidine formed with the corresponding benzaldehyde.[336]

9.3. Removal

The classification of the protecting group of Cys is particularly complex because most of the protecting groups used can be removed either by oxidation to the disulfide bridge or by other mechanisms. The following classification has drawn up taking into account these other mechanisms but also indicating the conditions for the oxidative removal in each particular case.

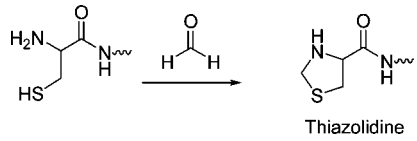


Figure 21. Thiazolidine formation by reaction of *N*-terminal Cys with formaldehyde.

9.3.1. Protecting Groups Removed by Acid (Table 20)

***p*-Methylbenzyl (Meb).**[337] More acid-labile than the Bn, it is removed with HF and scavengers at low temperatures.[338,339] It is gradually replacing the Bn in the Boc/Bn solid-phase strategy. It can also be removed with Tl(III) trifluoroacetate or with $MeSiCl_3$ in the presence of diphenylsulfoxide to yield disulfide bridges. However, p-methoxybenzyl (Mob) is usually a cleaner option.[340]

***p*-Methoxybenzyl (Mob).**[337] It is more acid-labile than Meb and is also used in the Boc/Bn solid-phase strategy. However, it is partially removed in the repetitive treatments to remove the Boc group when long peptide sequences are synthesized.[337] It is completely removed by HF at 0 °C and scavengers, TFMSA/TFA[341] and Hg (II) acetate or trifluoroacetate in TFA or AcOH, respectively.[342] It can be selectively removed in the presence of Meb using Ag(I) trifluoromethanesulfonate in TFA.[343] An intramolecular disulfide bridge between two Cys(Mob)-protected residues can be formed by removing the Mob group with $MeSiCl_3$ or $SiCl_4$ in TFA in the presence of diphenyl sulfoxide at 4 °C in 30 min.[344] In addition, oxidative removal with Tl(III) trifluoroacetate also leads to the formation of a disulfide bridge by reaction with a free Cys side chain.

Trityl (Trt).[345] It is removed with TFA and scavengers, such as triisopropylsilane (TIS) to prevent retritylation, or $AgNO_3$.[346] It is used for the Fmoc/^{t}Bu strategy, although Fmoc-Cys(Trt) can undergo racemization in basic carboxyl activation conditions.[331] It can also be removed by oxidation with iodine, thereby leading to a dilulfide bridge by reaction with a free Cys side chain. Other oxidative removals are listed in Table 20.[347]

Monomethoxytrityl (Mmt).[348] It is removed with diluted TFA and scavengers. It is considerably more acid-labile than the *S*-trityl group and can be removed selectively in its presence as well as in the presence of ^{t}Bu-protecting groups. Oxidative removal is similar to the case of the Trt group.

Trimethoxybenzyl (Tmob).[349] It is another more acid-labile alternative to the Trt group for the Fmoc/^{t}Bu strategy. It is removed with diluted TFA (5−30%) and scavengers; however, the trimethoxybenzyl cation resulting from its cleavage can alkylate Trp residues.

9-Xanthenyl (Xan).[331] It has similar stability features to Mmt; thus, it can also be removed selectively in the presence of *S*-trityl and ^{t}Bu-protecting groups or Rink and PAL handles.

2,2,4,6,7-Pentamethyl-5-dihydrobenzofuranylmethyl (Pmbf).[350] It is a relatively new highly acid-labile protecting group (Fmoc/^{t}Bu chemistry). It is removed with TFA−TES−DCM (0.5:5:94.5) in 2 h to render the free thiol. Alternatively, treatment with I_2 yields the disulfide bridge. This protecting group has been successfully applied to obtain oxytocin.

Benzyl (Bn).[351] It is removed with HF at 25 °C or Na in liquid ammonia. However, although still used, it is being replaced by other benzyl derivatives that do not require such harsh conditions for their removal.

***tert*-Butyl (^{t}Bu) and 1-Adamantyl (1-Ada).**[352] Both are fully stable to TFA and can, therefore, be used in the Boc/Bn strategy. They are also quite stable to HF at low temperatures but cleaved at higher temperatures in the presence of scavengers.[334] They are also stable to Ag(I) trifluoromethanesulfonate in TFA,[343] which quantitatively removes the *S*-Mmt group, as well as to iodine oxidation. Other possible cleavage conditions are listed in Table 20.[342]

9.3.2. Protecting Groups Removed by Base (Table 21)

9-Fluorenylmethyl (Fm).[353] It is removed with base (i.e., 50% piperidine−DMF for 2 h or 10% piperidine−DMF overnight)[354] and is very stable to strong acids such as HF. It is used in the Boc/Bn solid-phase strategy. It can be removed on solid phase or in solution, thereby yielding a disulfide because of air oxidation unless reducing thiols are employed. It is resistant to oxidative cleavage with iodine or $Tl(TFA)_3$ of other Cys-protecting groups.[334]

2-(2,4-Dinitrophenyl)ethyl (Dnpe).[355] It is removed with bases such as piperidine−DMF (1:1) in 30−60 min, thereby yielding the disulfide bridge, or in the presence of β-mercaptoethanol to give the free thiol. It is a less sterically hindered alternative to the Fm group for the Boc/Bn strategy (specially suited to facilitate the cleavage of peptides with *C*-terminal Cys), stable to strong acids such as HF and oxidative conditions to form disulfide bridges with Acm (I_2 or $Tl(TFA)_3$ in TFA).

Benzyl (Bn). See the section on protecting groups removed by acid.

9-Fluorenylmethoxycarbonyl (Fmoc).[356] Only preliminary solution studies are available for Cys thiol protection with Fmoc. It seems to be more base-labile than the Fm group. It is removed with TEA in the presence of I_2 or benzenethiol in DCM to yield the corresponding disulfide. These removal conditions do not affect the N^{α}-Fmoc group.

9.3.3. Other Protecting Groups (Table 22)

Acetamidomethyl (Acm).[357,358] Removed by oxidative treatment with I_2 or $Tl(TFA)_3$ to form disulfide bonds or with Hg(II) and Ag(TFMSO)[343] to obtain the free thiol. It is compatible with both the Boc/Bn and Fmoc/^{t}Bu strategies. Nevertheless, it is partially removed with HF or even TFA depending on the scavengers used. [359,325] In the latter case, absence of water and use of TIS minimizes the removal.[360]

Phenylacetamidomethyl (PhAcm).[361] It is an analogue of Acm that can be removed in similar conditions and also by treatment with the enzyme penicillin aminohydrolase.

***tert*-Butylmercapto (S^{t}Bu).**[362] It is removed with thiols (benzenethiol, β-mercaptoethanol, or dithiothreitol),[363] Na_2SO_3 in AcOH,[364] or phosphines (PBu_3 or PPh_3 in CF_3CH_2OH).[365] It is compatible with the Boc and Fmoc strategies. It is partially removed with HF but completely stable to TFA and to bases like piperidine.[366]

3-Nitro-2-pyridinesulfenyl (Npys). It is removed by reducing thiols and phosphines to render the free thiol.[366] It is stable to TFA and HF, but it is not stable to the low−high cleavage protocol or to bases.[368] It is used in the Boc/Bn strategy mainly to obtain disulfide bonds by nucleophilic displacement by the thiol of a free Cys.[369]

2-Pyridinesulfenyl (S-Pyr).[370] It is used in the Boc/Bn strategy and is useful when orthogonal protection of unprotected fragments is required. Ligation of a free thiocarboxylic

Table 20. Cys-Protecting Groups Removed by Acid

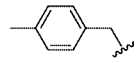
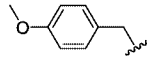
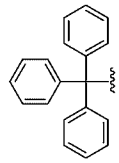
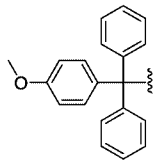
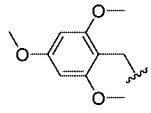
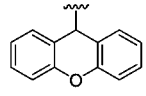
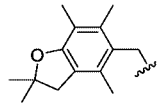
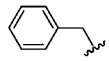
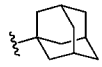
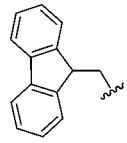
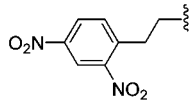
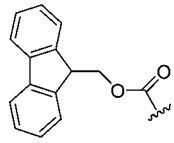
Name and Structure	Removal conditions (final S form)	Stability to the removal of	Compatibility with the most important sulfur protecting groups	Ref
p-Methylbenzyl (Meb) 	1) HF, scavengers **(SH)** 2) $MeSiCl_3$ or $SiCl_4$, TFA, Ph_2SO, **(S-S)** 3) $Tl(TFA)_3$ **(S-S)**	Boc, Fmoc, Trt, Alloc	Trt,[a] Acm, S^{t}Bu, Npys, Fm[b]	337, 338, 339, 340
p-Methoxybenzyl (Mob) 	1) HF, scavengers **(SH)** 2) TFMSA-TFA **(SH)** 3) $Hg(OAc)_2$ in TFA or $Hg(TFA)_2$, in AcOH **(SH)** 4) Ag (TFMSO) **(SH)** 5) $Tl(TFA)_3$ **(S-S)** 6) $MeSiCl_3$ or $SiCl_4$ TFA, Ph_2SO, 4°C, 30 min, **(S-S)**	Boc,[c] Fmoc, Trt, Alloc	Trt,[a] Acm, S^{t}Bu, Npys, Fm[b]	337, 341, 342, 343, 344
Trityl (Trt) 	1) 95% TFA, scavengers **(SH)** 2) $Hg(OAc)_2$ **(SH)** 3) $AgNO_3$ **(SH)** 4) I_2 **(S-S)** 5) $Tl(TFA)_3$ **(S-S)**	Fmoc, Alloc	Meb/Mob,[a] Acm,[d] S^{t}Bu, Npys, Fm[b]	331, 345, 346, 347
Monomethoxytrityl (Mmt) 	1) 1% TFA, scavengers **(SH)** 2) $Hg(OAc)_2$ **(SH)** 3) $AgNO_3$ **(SH)** 4) I_2 **(S-S)** 5) $Tl(TFA)_3$ **(S-S)**	Fmoc, Alloc	Meb/Mob,[a] Acm,[d] S^{t}Bu, Npys, Fm[b]	348
Trimethoxybenzyl (Tmob) 	5-30% TFA, scavengers **(SH)** 2) I_2 **(S-S)** 3) $Tl(TFA)_3$ **(S-S)**	Fmoc, Alloc		349
9-Xanthenyl (Xan) 	1% TFA , scavengers **(SH)**	Fmoc, Alloc		331
2,2,4,6,7-pentamethyl-5-dihydrobenzofuranylmethyl (Pmbf) 	1) TFA-TES-DCM (0.5-5-94.5) in 2 h **(SH)** 2) I_2 **(S-S)**	Fmoc		350

Table 20. (Continued)

Name and Structure	Removal conditions (final S form)	Stability to the removal of	Compatibility with the most important sulfur protecting groups	Ref
Benzyl (Bn) 	1) HF **(SH)** 2) Na, NH_3 **(SH)**	Boc, Fmoc, Trt, Alloc		351
tert-Butyl (^{t}Bu) 	1) HF (20°C) scavengers **(SH)** 2)TFMSA-TFA and scavengers **(SH)** 3) $Hg(OAc)_2$ in TFA **(SH)**	Boc, Fmoc, Trt, Alloc		334, 342, 343, 352
1-Adamantyl (1-Ada) 	1) HF (20°C) scavengers **(SH)** 2)TFMSA/TFA and scavengers **(SH)** 3)$Hg(OAc)_2$ in TFA **(SH)**	Boc, Fmoc, Trt, Alloc		334, 342, 343, 352

[a] The Trt group should be removed first. [b] The Fm should be removed first. [c] Except for repetitive treatments. [d] Trt should be removed first with TFA solutions.

Table 21. Cys-Protecting Groups Removed by Base

Name and Structure	Removal conditions	Stability to the removal of	Compatibility with other sulfur protecting groups	Ref
9-Fluorenylmethyl (Fm) 	1) 10-50% piperidine in DMF **(S-S)** 2) DBU in DMF **(S-S)**	Boc, Z,[a] Trt, Alloc	Meb/Mob/Trt,[b] Acm,[b]	334, 353, 354
2-(2,4-Dinitrophenyl)ethyl (Dnpe) 	Piperidine:DMF (1:1) **(S-S)** in the presence of mercaptoethanol: **(SH)**	Boc, Z,[a] Trt, Alloc		355
9-Fluororenylmethoxycarbonyl (Fmoc) 	TEA-benzenethiol or I_2 **(S-S)**	Boc, Z, [a] Trt, Alloc		356

[a] Except catalytic hydrogenolysis. [b] The Fm should be removed first.

peptide with an S-Pyr-protected *N*-terminal Cys occurs at pH 2, the subsequent *S*-to-*N* migration occurs at pH 7, and final treatment with DTT renders the final ligated peptide with free Cys. S-Pyr is stable to 1 M TFMSA in TFA−anisole (10:1) at 0 °C for 2 h (cleavage conditions for the MBHA resin).

Table 22. Other Cys-Protecting Groups Removed by Acid

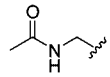
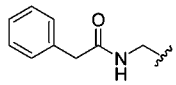
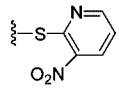
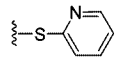
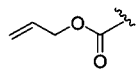
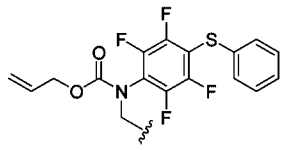
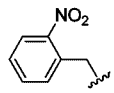
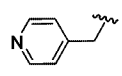
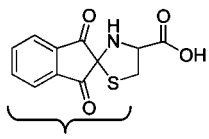
Name and Structure	Removal conditions	Stability to the removal of	Compatibility with other sulfur protecting groups	Ref.
Acetamidomethyl (Acm) 	1) I_2 **(S-S)** 2) $Tl(TFA)_3$ **(S-S)** 3) Ag(TFMSO) **(SH)** 4) Hg (II) **(SH)**	Boc, Fmoc, Alloc	Meb/Mob, Trt,[a] S^{t}Bu, Npys, Fm,[b] PhAcm[c]	325, 343, 357, 358, 359, 360
Phenylacetamidomethyl (PhAcm) 	1) Hg (II) **(SH)** 2) penicillin aminohydrolase **(SH)** 3) Tl (III) trifluoroacetate **(S-S)** 4) I_2 **(S-S)**	Boc, Fmoc, Z,[d] Alloc	Meb/Mob, S^{t}Bu, Npys, Fm[b], Acm[c]	361
5-tert-Butylmercapto (S^{t}Bu) 	1) thiols (benzenethiol, β-mercaptoethanol or dithiothreitol) 2) Na_2SO_3 in AcOH 3) PBu_3 or PPh_3 in CF_3CH_2OH	Boc, Fmoc, Trt	Meb/Mob, Trt, Acm	362, 363, 364, 365, 366
3-Nitro-2-pyridinesulfenyl (Npys) 	1) Thiol exchange with a free Cys **(S-S)** 2) Reducing thiols **(SH)** 3) PBu_3 (1 eq.), H_2O **(SH)**	Boc, Z,[d] Trt, Alloc	Meb/Mob, Trt, Acm	367, 368, 369
2-Pyridinesulfenyl (S-Pyr) 	Thiocarboxylic acids and DTT **(SH)**	Boc	Meb/Mob, Trt, Acm	370
Allyloxycarbonyl (Alloc) 	$Pd(PPh_3)_4$, Bu_3SnH **(SH)**	Boc, Trt		164
N-Allyloxycarbonyl-N-[2,3,5,6-tetrafluoro-4-(phenylthio)phenyl]] aminomethyl (Fsam) 	1) Pd (0), scavengers (i.e. $PdCl_2(PPh_3)_2$, Bu_3SnH or $Pd(PPh_3)_4$, $PhSiH_3$) 2) I_2 **(S-S)**			371
o-Nitrobenzyl (oNB) 	Photolysis (λ= 300-400 nm)	Boc, Fmoc, Trt, Z[e]		372, 373
4-Picolyl 	Zn dust in AcOH **(SH)**	Boc, Fmoc		374

Table 22. (Continued)

Name and Structure	Removal conditions	Stability to the removal of	Compatibility with other sulfur protecting groups	Ref.
Ninhydrin (Nin)  ***Nin***	1) 1 M Cys-OMe, 1 M DIPEA in DMF (solid phase) 2) 10% TFA in H_2O and Zn dust (solution) 3) Reducing thiols such as Cys in combination with TCEP (solution)	Z,[d] Boc		375

[a] The Trt should be removed first with TFA solutions. [b] The Fm should be removed first. [c] The PhAcm should be removed first enzymatically. [d] Except catalytic hydrogenation removal. [e] HF/anisole removal.

Allyloxycarbonyl (Alloc).[164] It is removed with tributyltin hydride catalyzed by Pd(0) (usually $Pd(PPh_3)_4$). Because of its base lability, it is used only in the Boc/Bn solid-phase strategy.

***N*-Allyloxycarbonyl-*N*-[2,3,5,6-tetrafluoro-4-(phenylthio)phenyl]aminomethyl (Fsam).**[371] It is an allyl-type protecting group that can be removed by palladium to render the free thiol both in solution and on solid phase, and it is the only Cys- protecting group that allows a selective and easy release of the thiol on solid phase. It is completely stable to TFA and piperidine and can also be removed by iodine oxidation to render a disulfide bridge.

***o*-Nitrobenzyl (*o*NB).**[372,373] It is a protecting group removed by photolysis ($\lambda = 300-400$ nm) and is used mainly in the synthesis of caged peptides.

4-Picolyl.[374] It is removed in solution with Zn dust in AcOH to render the free thiol. It was initially proposed for the Boc/Bn strategy but more recently has been successfully applied to the Fmoc/^{t}Bu synthesis of dihydrooxytocin, which was further oxidized to oxytocin.

Ninhydrin (Nin).[375] It has been proposed as a protecting group for *N*-terminal Cys. It protects both the amino and the thiol groups by forming a thiazolidine. Stable to HF and TFA, it is removed with 1 M Cys-OMe, 1 M DIPEA in DMF for 30 min (solid phase), 10% TFA in H_2O and Zn dust (solution), and reducing thiols such as Cys in combination with *tris*-carboxymethylphosphine (TCEP) (solution). It is coupled to amines linked to the solid phase without using further protection at the amino group. Its main applications are in ligation and its combination with His(Bom) in the Boc/Bn strategy, which prevents thiazolidine formation after Bom removal (see His protection).

The mercaptopropionic acid (des-amino Cys), which acts as an *N*-terminal capping in some peptides of therapeutic interest, can be introduced as a dimer. The free thiol is obtained after reduction with β-mercaptoethanol or Bu_3P.[376]

10. Methionine (Met)

10.1. General

The thioether funcionality of Met (Figure 22) can undergo two side reactions, oxidation to sulfoxide and *S*-alkylation.

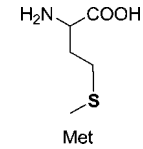


Figure 22. Methionine (Met).

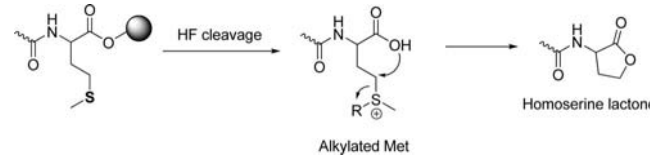


Figure 23. Homoserine lactone formation after Met alkylation during HF cleavage in the Boc/Bn solid-phase strategy.

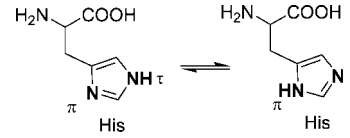


Figure 24. Histidine (His) tautomers.

The latter can lead to the formation of homoserine lactone in *C*-terminal Met (Figure 23).[377] These side reactions are favored in acidic conditions.

In the Fmoc/^{t}Bu strategy, Met is used unprotected in most of the cases. To prevent oxidation during amino acid side-chain deprotection and cleavage from the resin, ethylmethylsulfide or thioanisole are used.[378,379]

In contrast, in the Boc/Bn strategy, free Met may not be the best option because of the strong acidic conditions applied mainly in the cleavage from the resin but also in the removal of the Boc group. Therefore, very frequently, N^{α}-Boc-protected Met sulfoxide is directly used and is reduced at the end of the synthesis.

10.2. Introduction of the Protecting Groups

The sulfoxide derivatives of Met are commercially available and can be prepared via oxidation with H_2O_2.[380]

10.3. Removal: Sulfoxide Reduction

In the case of SPS, the reduction of Met(O) can be performed either during the cleavage or after it. In the latter

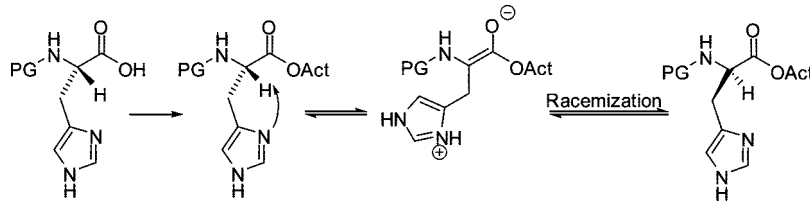


Figure 25. Proposed racemization mechanism of His during the coupling step .

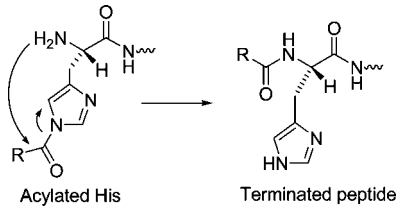


Figure 26. N^{τ} to α-amino migration after acylation of His during peptide synthesis.

case, the sulfoxide functionality confers extra polarity to protected peptides, which facilitates its purification; however, it must be taken into consideration that sulfoxides are chiral, and therefore, different diastereomers will be observed.

Several reduction methods have been used:

(1) Reduction during the low−high HF or TFMSA cleavage in the Boc/Bn strategy. DMS and *p*-thiocresol or anisole should be used as scavengers to prevent *S*-alkylation.

(2) *N*-Methylmercaptoacetamide in 10% aquous acetic acid.[381−383] It requires long reaction times, and disulfide bridges may be reduced.

(3) TFA−NH_4I−DMS.[384−386] This method of reduction does not affect disulfide bridges, and if there are free Cys residues, a disulfide bridge is formed during the reduction of the Met sulfoxide. *tert*-Butyl-type groups are removed during the reduction. Dimerization of Trp (see Trp section) can occur in the case of long reaction times as a result of overexposure to acidic conditions.

(4) $TiCl_4$(3 equiv)−NaI(6 equiv) in MeOH−acetonitrile−DMF (5:5:4).[387] Although a very fast reduction method, it can also lead to reduction of disulfide bridges or oxidation of Trp, with the latter caused by the I_2 generated in the sulfoxyde reduction.

(5) TFA−TMSBr−EDT.[388,389] In this method, the reduction is carried out by addition of TMSBr and EDT at the end of the cleavage step. It appears to be compatible with Trp- containing peptides. The peptide is isolated by precipitation in diethylether.

(6) Bu_4NBr in TFA. It is an alternative to method 5 in which the reduction is also carried out during the cleavage step.[390]

(7) Sulfur trioxide (5 equiv), EDT (5 equiv) in pyridine−DMF (2:8).[391] In this method, protection of hydroxyl groups is required to prevent sulfonylation.

Met des-tert-butylation

If *tert*-butylation occurs during the global deprotection step, reversion to the free Met residue is accomplished by heating a solution of the peptide in 4% $AcOH_{(aq)}$ at 60−65 °C.[392,393]

11. Histidine (His)

11.1. General

The imidazole ring of His (Figure 24) has two nucleophilic points, the π- and τ-nitrogens.[394]

Unprotected His is highly prone to racemization during the coupling (Figure 25) and acylation during peptide synthesis followed by N^{τ} to α-amino migration (Figure 26).[395,396]

The basic and nucleophilic π-nitrogen is the one involved in racemization mechanisms and can be masked in two ways: (i) direct protection and (ii) τ-nitrogen protection with bulky or electron-withdrawing protecting groups, which reduce the basicity of the π-nitrogen.

Although a large number of protecting groups have been tested for His side-chain protection, either in the π- or τ-nitrogen, the problem has still not been fully resolved, with the situation being more critical in the case of the Boc/Bn solid-phase strategy.

The most used protecting groups are Trt for the Fmoc/^{t}Bu solid-phase strategy and Dnp (2,4-dinitrophenyl), Bom (benzyloxymethyl), and Tos (tosyl) for the Boc/Bn solid-phase strategy.

11.2. Introduction of the Protecting Groups[394]

Protection of the imidazole ring of His requires α-amino and carboxylic acid protection with orthogonal protecting groups. In cases such as Trt, the α-amino group can be used unprotected and at the end of the synthesis the N^{α}-trityl is removed, thereby leaving the N^{im}-trityl unalterated. Generally, the reaction of the imidazole ring of His with the corresponding active species (halides in general) gives the N^{τ}-protected imidazole as a majority and sometimes single product. Nevertheless, N^{π} protection is preferred because, as previously mentioned, the N^{π} is the one directly involved in His racemization. Thus, when possible, N^{π} protection is performed by masking the τ-nitrogen with an orthogonal protecting group, which is removed at the end of the synthesis of the derivative.

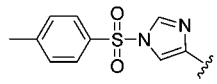
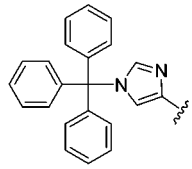
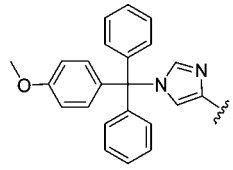
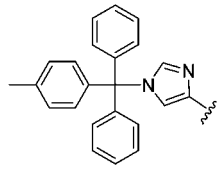
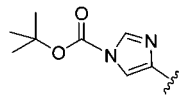
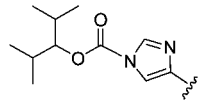
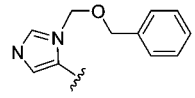
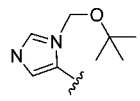
11.3. Removal

11.3.1. Protecting Groups Removed by Acid (Table 23)

11.3.1.1. N^{τ}-Tosyl (Tos).[397] It is removed with HF. It minimizes racemization by reducing the basicity of the N^{π} by inductive effect and also because of steric hindrance. Although it is still quite commonly used in the Boc/Bn solid-phase strategy, it is unstable in the presence of N^{α} groups and HOBt.[398,395]

N^{τ}-Trityl (Trt). It is the usual protecting group for the Fmoc/^{t}Bu strategy.[21,399] It is removed with 95% TFA but is much less acid-labile than the N^{α}-trityl group and cannot be selectively removed in the presence of ^{t}Bu groups.[400] Using N^{τ} protection, the free N^{π} can still catalyze racemization. However, the bulkiness of the Trt group minimizes this side

Table 23. His-Protecting Groups Removed by Acid

Name and Structure	Removal conditions	Stability to the removal of	Ref
N^{τ}-Tosyl (Tos) 	HF, scavengers	Boc, Trt	395, 397, 398
N^{τ}-Trityl (Trt) 	95% TFA	Fmoc, Alloc.	21, 394, 399, 400
N^{τ}-Monomethoxytrityl (Mtt) 	15 % TFA, DCM, 1 h	Fmoc, Alloc	400
N^{τ}-Methyltrityl (Mmt) 	5 % TFA, DCM, 1 h	Fmoc, Alloc	400
N^{τ}-tert-Butyloxycarbonyl (Boc) 	TFA, scavengers	Fmoc,[a] Alloc	399
N^{τ}-2,4-Dimethylpent-3-yloxycarbonyl (Doc) 	HF, scavengers	Boc, Z,[b] Trt	401
N^{π}-Benzyloxymethyl (Bom) 	1) HF, scavengers 2) TFMSA-TFA 3) Catalytical Hydrogenation	Boc, Fmoc,[c] Trt	326, 327, 402
N^{π}-tert-Butoxymethyl (Bum) 	TFA, scavengers	Fmoc, Z[b]	403, 404

[a] Only stable to a few Fmoc removal cycles (partially labile to piperidine). [b] Catalytic hydrogenation removal. [c] Except catalytic hydrogenation removal.

reaction in most cases, but it is still critical in particular cases such as the formation of ester bonds or when the amino component is sterically hindered.[394]

N^{τ}-Methyltrityl (Mtt) and N^{τ}-monomethoxytrityl (Mmt). These are more acid-labile derivatives of the Trt group; they are removed with 15% and 5% TFA in DCM in 1 h. [400]

N^{τ}-tert-Butyloxycarbonyl (Boc). It is only useful for the synthesis of short sequences via Fmoc chemistry because of its instability to prolonged piperidine treatments.[399] Its slightly greater acid stability compared with Trt makes it highly suitable for the preparation of His-containing protected peptides using a ClTrtCl resin.

Table 24. His-Protecting Groups Removed by Base

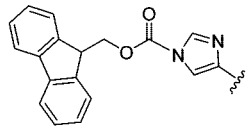
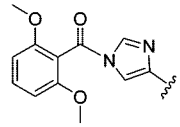
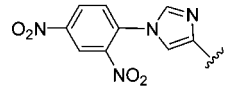
Name and Structure	Removal conditions	Stability to the removal of	Ref
9-Fluorenylmethoxycarbonyl (Fmoc) 	Piperidine-DMF (2:8)	Boc	234, 405
2,6-Dimethoxybenzoyl (Dmbz) 	1) 32% $NH_{3\,(aq)}$-dioxane (1:1), 6 h. 2) 32% $NH_{3\,(aq)}$-EtOH (3:1), 2 h.	Boc, Fmoc, Trt	406

Table 25. Other His-Protecting Groups

Name and Structure	Removal conditions	Stability to the removal of	Ref.
N^{τ}-2,4-Dinitrophenyl (Dnp) 	Thiolysis (e.g. thiophenol, DBU)	Boc, Z,[a] Trt	407,408, 409,410, 411

[a] Except catalytic hydrogenation removal.

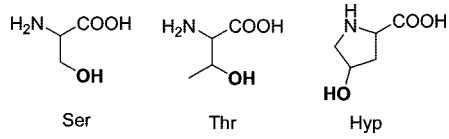


Figure 27. Serine (Ser), Threonine (Thr), and Hydroxyproline (Hyp).

N^{τ}-2,4-Dimethylpent-3-yloxycarbonyl (Doc).[401] It is removed with liquid HF and is used in the Boc/Bn sol-d phase strategy. In contrast to other proposed carbamate-type His-protecting groups, it is very resistant to nucleophiles because of its bulkiness, thereby preventing N^{im} to N^{α} transfer. It is not stable to 2% hydrazine in DMF but is more stable to piperidine than the 2,4-dinitrophenyl (Dnp) group (see section on other protecting groups) (its half-life in 20% piperidine in DMF is 84 h).

11.3.1.2. N^{τ}-Protection. *N^{τ}-Benzyloxymethyl (Bom).* It is removed by HF, TFMSA, or hydrogenolysis and is completely stable to bases and nucleophiles. It has been extensively used for the Boc/Bn solid-phase strategy. Because formaldehyde is released during Bom cleavage, appropiate scavengers should be used to prevent formylation, methylation, or the formation of thiazolidines when an *N*-terminal Cys is present.[326,327] In addition, a recent report shows that α-amino Boc removal of Bom-protected His requires harsher conditions than those commonly used.[402]

N^{π}-tert-Butoxymethyl (Bum).[403,404] It is removed by TFA and resistant to hydrogenolysis. Formylation during its removal can be prevented using appropiate scavengers in the same way as for Bom. It prevents racemization of His in the Fmoc/^{t}Bu strategy; however, it is not widely used because of the difficult synthesis of Fmoc-His(π-Bum)-OH.

11.3.2. Protecting Group Removed by Base (Table 24)

N^{τ}-9-Fluorenylmethoxycarbonyl (Fmoc)[405]

It is removed with piperidine−DMF (2:8) and has been used for the synthesis of peptide−oligonucleotide conjugates.[234]

N^{τ}-2,6-Dimethoxybenzoyl (Dmbz)[406]

It is a relatively recently developed protecting group for the Fmoc/^{t}Bu strategy, and therefore, it has not been widely used. Removed with ammonia solutions and stable to the removal of *tert*-butyl type groups, it minimizes His racemization during the coupling to the same extent as Trt and also reduces acyl migration.

11.3.3. Other Protecting Groups (Table 25)

N^{τ}-2,4-Dinitrophenyl (Dnp)[407]

It is removed by thiolysis[408,409] and is stable to HF. It is also commonly used in the Boc/Bn solid-phase strategy. However, it also has some drawbacks: incomplete removal can occur in sequences rich in His and it is labile to nucleophiles. These features makes it incompatible with Lys(Fmoc) because after Fmoc removal the Dnp group can migrate to the free amino of the Lys.[410] In addition, it must be removed before eliminating the last α-Boc group.[411]

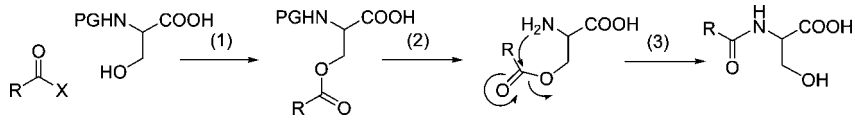
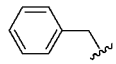
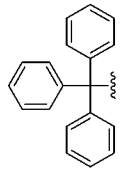
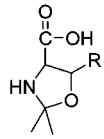


Figure 28. *O*-acylation followed by *O*−*N* migration after amino deprotection: (1) *O*-acylation, (2) amino-protecting group (PG) removal, and (3) *O*−*N* migration.

Table 26. Ser, Thr, and Hyp-Protecting Groups Removed by Acid

Name and Structure	Removal conditions	Stability to the removal of	Ref.
Benzyl (Bn) 	1) HF, scavengers 2) TFMSA-TFA	Boc, Fmoc, Trt, Alloc, *p*NZ[a]	423
Cyclohexyl (cHx) 	TFMSA-TFA	Boc, Fmoc, Trt, Alloc, *p*NZ	424
tert-Butyl (tBu) 	90% TFA-DCM	Fmoc, Z,[b] Alloc, *p*NZ	416, 425
Trityl (Trt) 	1% TFA-DCM	Fmoc, Alloc	421, 426
tert-Butyldimethylsilyl (TBDMS) 	1) TFA 2) AcOH-THF-H_2O (3:1:1), 18 h (Ser), 2 h (Thr) 3) 0.1 M TBAF in DMF, 2h (Ser), 18 h (Thr)	Fmoc	422
Pseudoprolines  R= H (Ser) or Me (Thr)	95% TFA and scavengers	Fmoc, Alloc	

[a] Except catalytic hydrogenation removal. [b] Catalytic hydrogenation removal.

12. Serine (Ser), Threonine (Thr), and Hydroxyproline (Hyp)

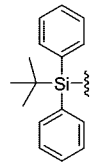
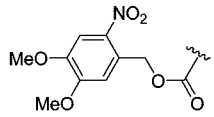
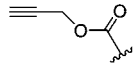
12.1. General

Amino acids containing unprotected hydroxyl functionalities such as Ser, Thr, and Hyp (Figure 27) can undergo side reactions such as dehydratation or *O*-acylation followed by *O*−*N* migration after amino deprotection (Figure 28).

Although the protected derivatives are the safest way to incorporate Ser, Thr, or Hyp into the peptide sequence, they can also be used with the free hydroxyl functionality. Protection is more necessary in SPS, because an excess of acylating agents is used, and for Ser, whose primary alcohol is more prone to acylation than the secondary alcohols of Thr and Hyp, which have been successfully used without protection in several syntheses, including solid phase.[412,413] Nevertheless, there are also some reports of the successful use of unprotected Ser in solution-phase synthesis, but care must be taken when choosing the activating agents.[414,415]

In peptide synthesis, hydroxyl functionalities are protected as ethers, which are more stable than the corresponding carbamates and esters. The most used protecting groups for the Boc/Bn and Fmoc/^{t}Bu strategies are Bn (benzyl) and ^{t}Bu (*tert*-butyl), respectively.

Table 27. Other Ser, Thr, and Hyp-Protecting Groups

Name and Structure	Removal conditions	Stability to the removal of	Ref.
***tert*-Butyldiphenylsilyl (TBDPS)** 	1) 1 M TBAF (2-3 eq.), THF, 1-5 h 2) 2 M $NaOH_{(aq)}$ EtOH (1:1), 7h	Boc, Fmoc, Trt	427, 428
4,5-Dimethoxy-2-nitrobenzyloxycarbonyl (Dmnb) 	Photolysis (visible blue light)	Boc, Fmoc, Trt	429, 430
Propargyloxycarbonyl (Poc) 	$[(PhCH_2NEt_3)_2MoS_4]$ in AcCN, 1h.	Boc, Fmoc, Trt	434

12.2. Introduction of the Protecting Groups

Distinct protection methods are used depending on the kind of protecting group. ^{t}Bu protection is carried out via addition of isobutylene in acidic conditions.[416] Bn protection is performed using benzyl bromide in basic conditions in the case of Ser,[417,418] and reaction with benzyl alcohol in acidic medium in the case of Thr.[419]

Bn and ^{t}Bu protections can also be achieved via formation of 2,2-difluoro-1,3,2-oxazaborolidin-5-ones by reaction of the lithium salt of Ser or the sodium salt of Thr with BF_3. Treatment with isobutylene (^{t}Bu protection) or benzyl 2,2,2-trichloroacetimidate (Bn protection) followed by a base treatment to destroy the 2,2-difluoro-1,3,2-oxazaborolidin-5-one generates the desired protected derivatives.[420] Trt and alkylsilane protection are achieved using the respective chlorides in the presence of a base.[421,422]

12.3. Removal

12.3.1. Protecting Groups Removed by Acid (Table 26)

Benzyl (Bn).[423] It is removed with HF in the presence of scavengers and is the most used protecting group for Ser and Thr in the Boc/Bn solid-phase strategy. When many benzyl ethers are present, appropriate scavengers should be used to avoid benzylation of free amino acid side chains.

Cyclohexyl (cHx).[424] It is an alternative to the benzyl group for the protection of Ser in the Boc/Bn solid-phase strategy. It is more stable to acids and completely stable to catalytic hydrogenation. However, it has not been widely used.

***tert*-Butyl (^{t}Bu).[416]** It is removed with TFA and used mainly in the Fmoc/^{t}Bu solid-phase strategy. ^{t}Bu ethers are less acid-labile than the Boc group, and some reports indicate that they can be used even as temporary protecting groups in the Boc/Bn solid-phase strategy.[425]

Trityl (Trt).[421] It is removed with 1% TFA. It has been shown that the same peptide with all the hydroxyl groups protected by Trt or ^{t}Bu is obtained with better purity in the case of the former.[426]

***tert*-Butyldimethylsilyl (TBDMS).[422]** It is more acid-labile than the ^{t}Bu group and can be removed selectively in the presence of this group using AcOH−THF−H_2O (3:1:1) or TBAF.

Pseudoprolines. See the section on amide backbone protection.

12.3.2. Other Protecting Groups (Table 27)

***tert*-Butyldimethylsilyl (TBDMS).** See the section on protecting groups removed by acid.

***tert*-Butyldiphenylsilyl (TBDPS).[427,428]** It is typically removed by TBAF but also by 2 M $NaOH_{(aq)}$−EtOH (1:1). It is more acid-stable than TBDMS and stable to the removal of *N*-Trt, *O*-Trt, *O*-TBDMS, and Boc.

4,5-Dimethoxy-2-nitrobenzyloxycarbonyl (Dmnb).[429] It is a photolabile protecting group analogous to the corresponding Dmnb ester. Ser(Dmnb) has been used recently to control protein phosporyltion.[430]

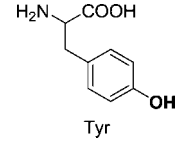
Propargyloxycarbonyl (Poc).[431] It is removed with $[(PhCH_2NEt_3)_2MoS_4]$ in AcCN, 1 h, rt. These removal conditions do not affect Boc, Z, methyl, or benzyl esters. It has recently been applied to the protection of Ser and Thr for peptide synthesis in solution.

13. Tyrosine (Tyr)

13.1. General

Use of unprotected Tyr (Figure 29) can lead to acylation of the phenol group because of the nucleophilicity of the phenolate ion under basic conditions. In addition, the electron-rich aromatic ring can undergo alkylation at the ortho position.

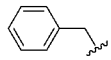
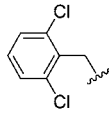
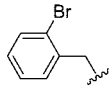
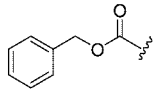
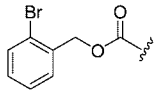
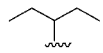
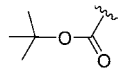
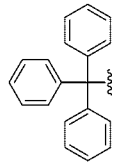
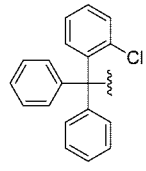
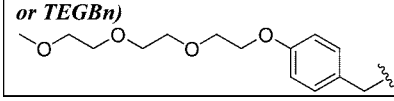
The acidity of the phenol group makes alkyl-type protecting groups less stable than in the case of Ser, Thr, and Hyp.



Tyr

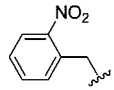
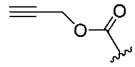
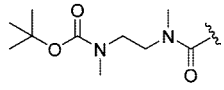
Figure 29. Tyrosine (Tyr).

Table 28. Tyr-Protecting Groups Removed by Acid

Name and Structure	Removal conditions	Stability to the removal of	Ref.
Benzyl (Bn) 	1) HF and scavengers 2) H_2 cat.	Boc, Fmoc, Trt, Alloc, *p*NZ [a]	27, 434
tert-Butyl (^{t}Bu) 	35% TFA-DCM	Fmoc, Z,[b] Alloc, Trt, *p*NZ	
2,6-Dichlorobenzyl (Dcb) 	HF and scavengers	Boc, Fmoc, Trt, Alloc, *p*NZ[a]	432
2-Bromobenzyl (BrBn) 	HF and scavengers	Boc, Fmoc, Trt, Alloc, *p*NZ[a]	435
Benzyloxycarbonyl (Z) 	HF and scavengers	Boc, Trt	337
2-Bromobenzyloxycarbonyl (BrZ) 	HF and scavengers	Boc, Trt	432, 433, 436
3-Pentyl (Pen) 	HF and scavengers	Boc, Fmoc, Z,[b] Trt	437
tert-Butyloxycarbonyl (Boc) 	TFA-DCM		438
Trityl (Trt) 	2% TFA-DCM	Fmoc, Alloc	293, 421, 426, 439
2-Chlorotrityl (2-Cl-Trt) 	2% TFA in DCM	Fmoc, Alloc	293, 421, 426, 439
tert-Butyldimethylsilyl (TBDMS) 	1) 35% TFA 2) 0.1 M TBAF-DMF, 15 min.	Fmoc	422
4-(3,6,9-Trioxadecyl)oxybenzyl (TEGBz or TEGBn) 	TFA-DCM	Fmoc, Trt	

[a] Except catalytic hydrogenation removal. [b] Catalytic hydrogenation removal.

Table 29. Other Tyr-Protecting Groups

Name and Structure	Removal conditions	Stability to the removal of	Ref.
Allyl (Al) 	$Pd(Ph_3)_4$, scavengers	Boc, Fmoc, Z[a]	98, 164, 440
o-Nitrobenzyl (oNB): 	Photolysis (λ=350 nm),12 h	Boc, Fmoc, Trt	441, 442
Propargyloxycarbonyl (Poc) 	$[(PhCH_2NEt_3)_2MoS_4]$ in AcCN, 1h.	Boc, Trt	431
Boc-N-Methyl-N-[2-(methylamino)ethyl]carbamoyl (Boc-Nmec) 	i) 25-50% TFA ii) *N*-methylmorpholine (10 eq) in DMF/H_2O (3:7), 4 h	Fmoc, Trt	443

[a] Except catalytic hydrogenation removal.

The most used Tyr-protecting groups for the Boc/Bn and Fmoc/^{t}Bu solid-phase strategies are Bn and ^{t}Bu group, respectively.

13.2. Introduction of the Protecting Groups

To protect the phenolic function of Tyr,[345] both the amino and carboxylic groups must be protected by either forming a copper(II) chelate or using orthogonal protecting groups.

^{t}Bu-protected Tyr is obtained using isobutylene in acidic medium,[416] whereas with the other Tyr-protected derivatives, the corresponding alkyl halide is used as the protecting agent.[432,433]

13.3. Removal

13.3.1. Protecting Groups Removed by Acid (Table 28)

Benzyl (Bn). It is removed with HF but can lead to benzylation of the aromatic ring of Tyr, and it is not stable enough to the repetitive treatments with 50% TFA in DCM to remove the Boc group.[434] Milder removal conditions for the Boc group allow the synthesis of long peptides using benzyl protection.[27] In solution synthesis, it is usually removed by catalytic hydrogenation.

***tert*-Butyl (^{t}Bu).** It is removed with TFA and is the most used protecting group for the Fmoc/^{t}Bu strategy solid-phase strategy. It is more stable than the *tert*-butyl ethers of Ser, Thr, and Hyp. It is also stable to fluoride ions (TBAF).

2,6-Dichlorobenzyl (Dcb).[432] It is removed with HF, and because of its major acid stability, it is an alternative to the Benzyl group for the Boc/Bn solid-phase strategy.

2-Bromobenzyl (BrBn).[435] It is another more acid-stable derivative of the benzyl group; however, it has not found as wide application as Dcb.

Benzyloxycarbonyl (Z).[337] It is removed with HF and protects the phenol functionality by forming a carbonate. Although still used, it is too acid-labile to withstand repetitive treatments with 50% TFA to remove the Boc group.

2-Bromobenzyloxycarbonyl (BrZ).[432,433] It protects the phenol functionality by forming a carbonate, but unlike with other carbonates, only minor amounts of *O*-to-*N* transfer are observed. In contrast to the above-mentioned Z group, BrZ is very stable to acidic conditions (removed with HF) and widely used for the SPS of long peptides using the Boc/Bn solid-phase strategy.[432,433] It cannot be used in the Fmoc/^{t}Bu strategy because, being a carbonate, it is very sensitive to bases and nucleophiles.[436]

3-Pentyl (Pen).[437] It is a relatively new protecting group, stable to 50% TFA, bases, and catalytic hydrogenation, and readily removed with HF.

***tert*-Butyloxycarbonyl (Boc).[438]** This carbonate has been used occasionally for Tyr side-chain protection in the Boc/Bn solid-phase strategy but only protects the phenol during the coupling and is removed with TFA along to N^{α}-Boc.

Trityl (Trt) and 2-Chlorotrityl (2-Cl-Trt). They are very acid-labile and have the advantage of the low electrophilicity of trityl cations. Thus, they are a better alternative to ^{t}Bu for the synthesis of peptides containing residues prone to alkylation such as Trp and Met.[421,439,426] Removal is carried out with 2% TFA in DCM.[293]

***tert*-Butyldimethylsilyl (TBDMS).[422]** Unlike the ^{t}Bu ethers, the TBDMS ether of Tyr is more acid-labile than the corresponding ^{t}Bu ethers; however, it can be removed selectively with TBAF.

4-(3,6,9-Trioxadecyl)oxybenzyl (TEGBz or TEGBn). See section 5.3.1.

13.3.2. Other Protecting Groups (Table 29)

Benzyl (Bn). See section on protecting groups removed by acid.

***tert*-Butyldimethylsilyl (TBDMS).** See section on protecting groups removed by acid.

Allyl (Al).[440,98,164] Removed with Pd(0), it is strictly orthogonal to the most common protecting groups. It is used in both solution strategies and SPS.

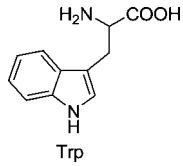


Figure 30. Tryptophan (Trp).

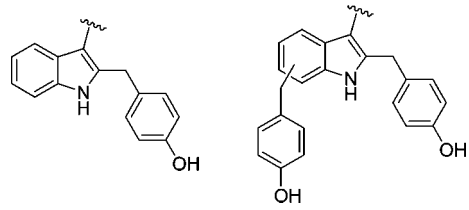


Figure 31. Alkylation of Trp by the Wang linker side products.

***o*-Nitrobenzyl (*o*NB).**[441] A photolabile protecting group, it has the same properties as the *o*NB ester. It has been used for the synthesis of Tyr caged peptides. [442]

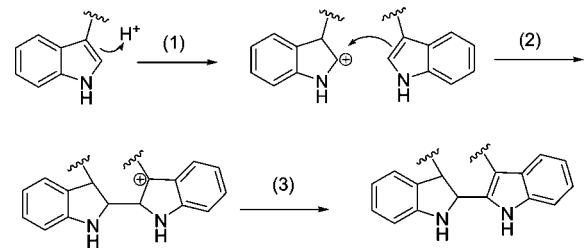
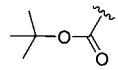
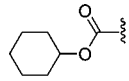
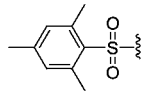


Figure 32. Mechanism of Trp dimerization: (1) protonation, (2) nucleophilic attack, and (3) elimination.

Propargyloxycarbonyl (Poc).[431] It is removed with $[(PhCH_2NEt_3)_2MoS_4]$ in AcCN, 1 h, rt. These removal conditions do not affect Boc, Z, methyl, or benzyl esters. It has recently been applied to the protection of Tyr for peptide synthesis in solution.

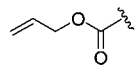
Boc-*N*-Methyl-*N*-[2-(methylamino)ethyl]carbamoyl (Boc-Nmec).[443] It is a recently developed protecting group (see also Boc-Nmec-Hmb in section 6.3.2). After removal

Table 30. Trp-Protecting Groups Removed by Acid

Name and Structure	Removal conditions	Stability to the removal of	Ref.
Formyl (For) 	1) Strong acid (HF) and scavengers (i.e. EDT) (slow) 2) piperidine-H_2O or DMF 3) 1 M NH_2OH, pH 9, 2h	Boc	457, 458, 459, 460
tert-Butyloxycarbonyl (Boc) 	95% TFA and scavengers [a]	Fmoc, Alloc	453, 454, 461, 462
Cyclohexyloxycarbonyl (Hoc) 	HF, scavengers	Boc, Fmoc, Alloc,	452, 463, 464
Mesityl-2-sulfonyl (Mts) 	1) CF_3SO_3H/TFA 2) $MeSO_3H$	Boc, Fmoc, Alloc,	465, 466

[a] The carbamic acid resulting from *tert*-butyl removal is quite stable. Complete decarboxylation takes place by treatment with 0.1 M $AcOH_{(aq)}$ or more slowly during lyophilization in H_2O.

Table 31. Other Trp-Protecting Groups

Name and Structure	Removal conditions	Stability to the removal of	Ref.
Allyloxycarbonyl (Alloc) 	$Pd(PPh_3)_4$, methylanylin in DMSO-THF-0.5 M HCl (1:1:0.5), 8h	Boc	455

of the Boc group, the Nme moiety is removed with *N*-methylmorpholine (10 equiv) in DMF/H_2O (3:7), 4 h.

14. Tryptophan (Trp)

14.1. General

The indole group of Trp (Figure 30) can undergo oxidation and alkylation if it is not protected.[444]

Alkylation during acid treatments can be done by carbocations from released protecting groups or from the resin, with the latter leading to irreversible bonding of the peptide to the support (Figure 31).[445]

Dimerization of Trp caused by alkylation by another protonated Trp has also been observed (Figure 32).[446,447]

In the Boc/Bn strategy, the higher risk of oxidation and alkylation in acidic media makes the protection of Trp necessary. In addition, care must be taken when chosing the scavengers in the final cleavage. For instance, thioanisole should be avoided because thioanisole cation adducts can alkylate Trp, and TIS, which is mainly used in the Fmoc/^{t}Bu strategy, should be used instead of TES to prevent reduction of the indole ring of Trp to indoline.[448] The most used protecting group for the Boc/Bn strategy is For (formyl).

In contrast, in the Fmoc/^{t}Bu strategy, unprotected Trp is often used. However, in many cases, protection is necessary. A critical example is when the peptidic sequences contain Arg protected by either Mtr, Pmc, or Pbf groups, which after removal can react with the indole ring in the 2 position.[449,450] The most used protecting group for the Fmoc/^{t}Bu strategy is Boc.

14.2. Introduction of the Protecting Groups

Carbamate protection of the *tert*-butyl, benzyl, or phenacyl esters of N$^\alpha$ Boc or Z-Trp is easily carried out using di-*tert*-butyldicarbonate or an appropiate chloroformate in the presence of a tertiaty base. After that, the carboxylic acid and/or amino-protecting groups are removed and N^α derivatization yields the Boc and Fmoc derivatives of the protected Trp.[451−455] The formyl group is introduced using an excess of formic acid.[456]

14.3. Removal

14.3.1. Protecting Groups Removed by Acid (Table 30)

Formyl (For).[457] Removal with HF may be slow, and the use of thiols (i.e., EDT) as scavengers makes it faster[458] In the case of base cleavage, care must be taken with the reaction conditions in order to avoid free amine formylation.[459,460]

***tert*-Butyloxycarbonyl (Boc).**[453,454] It is removed with high concentrations of TFA and is the protecting group of choice for the Fmoc/^{t}Bu solid phase strategy. It is more stable than Boc α-amino protection, which can be removed in the presence of protected Trp if care is taken with the reaction conditions, but not as a routine procedure. Boc protection avoids Trp alkylation during the removal of Mtr, Pmc, and Pbf from the Arg side chain.[461,462] The *N*-carboxylated compound can be detected after *tert*-butyl removal but later becomes unstable, thereby giving the free indole. The stability of this carbamic acid makes Boc-protected Trp less prone to electrophilic additions during the final cleavage.[453,454]

Cyclohexyloxycarbonyl (Hoc).[452] It is an alternative to the formyl group for the Boc/Bn strategy. Its high resistance to bases makes it useful for the synthesis of protected peptides on base-labile resins.[463] Although it is generally removed with HF in the presence of *p*-cresol, Trp alkylation by *p*-cresol can occur during the removal. A proposed solution for this problem is the use of Fmoc-Leu or butanedithiol as scavengers.[464]

Mesityl-2-sulfonyl (Mts).[465] Another alternative for the Boc/Bn strategy, Mts is removed by 1 M CF_3SO_3H/TFA or $MeSO_3H$ but not by HF. Although it has not been widely applied, there are reports of its use.[466]

14.3.2. Protecting Groups Removed by Base

Formyl (For). See the section on protecting groups removed by acid.

14.3.3. Other Protecting Groups (Table 31)

Allyloxycarbonyl (Alloc).[455] Removed with Pd(0), its orthogonality to Boc and Fmoc (when removed with DBU but not when removed with piperidine) makes it potentially useful for both the Boc/Bn and the Fmoc/^{t}Bu solid-phase strategies.

15. Abbreviations

AB linker	3-(4-hydroxymethylphenoxy)propionic acid linker
Acm	acetamidomethyl
Ac	acetyl
1-Ada	1-adamantyl
Al	allyl
Alloc	allyloxycarbonyl
API	active pharmaceutical ingredients
Arg	arginine
Asn	asparagine
Asp	aspartic acid
Azoc	azidomethyloxycarbonyl
Bn	benzyl
BAL	backbone amide linker
Boc	*tert*-butyloxycarbonyl
Bom	benzyloxymethyl
Bpoc	2-(4-biphenyl)isopropoxycarbonyl
BrBn	2-bromobenzyl
BrPhF	9-(4-bromophenyl)-9-fluorenyl
BrZ	2-bromobenzyloxycarbonyl
Bsmoc	1,1-dioxobenzo[*b*]thiophene-2-ylmethyloxycarbonyl
Bum	*tert*-butoxymethyl
Cam	carbamoylmethyl
cHx	cyclohexyl
Cl-Z	2-chlorobenzyloxycarbonyl
Cpd	cyclopropyldimethylcarbinyl
Cys	cysteine
Dab	diaminobutyric acid
Dap	diaminopropionic acid
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
Dcb	2,6-dichlorobenzyl
DCHA	dicyclohexylammonium
DCM	dichloromethane
Dde	(1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)-3-ethyl)
Ddz	α,α-dimethyl-3,5-dimethoxybenzyloxycarbonyl
dio-Fmoc	2,7-diisooctyl-Fmoc
DIPEA	*N*,*N*-diisopropylethylamine
DKP	diketopiperazine
Dma	1,1-dimethylallyl
Dmab	4-(*N*-[1-(4,4-Dimedhyl-2,6-dioxocyclohexylidene)-3-methylbutyl]amino)benzyl
Dmb	2,4-dimethoxybenzyl
Dmcp	dimethylcyclopropylmethyl

DMF	*N*,*N*-dimethylformamide
Dmnb	4,5-dimethoxy-2-nitrobenzyl/oxycarbonyl
DMSO	dimethylsulfoxide
dNBS	2,4-dinitrobenzenesulfonyl
Dnp	2,4-dinitrophenyl
Dnpe	2-(2,4-dinitrophenyl)ethyl
Doc	2,4-dimethylpent-3-yloxycarbonyl
Dts	dithiasuccinoyl
DTT	dithiothreitol
$EDOT_n$	3,4-ethylenedioxy-2-thenyl
Esc	ethanesulfonylethoxycarbonyl
Fm	9-fluorenylmethyl
Fmoc	9-fluorenylmethoxycarbonyl
Fmoc(2F)	2-fluoro-Fmoc
Fmoc*	2,7-di-*tert*-butyl-Fmoc
For	formyl
Fsam	*N*-allyloxycarbony-*N*-[2,3,5,6-tetrafluoro-4-(phenylthio)phenyl]aminomethyl
Gln	glutamine
Glu	glutamic acid
HFA	hexafluoroacetone
His	histidine
Hmb	2-hydroxy-4-methoxybenzyl
Hoc	cyclohexyloxycarbonyl
HOBt	1-hydroxybenzotriazole
HOSu	*N*-hydroxysuccinimido
Hyp	hydroxyproline
ivDde	1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)-3-methylbutyl
Lys	lysine
Mbh	4,4′-dimethoxybenzhydryl
MBHA	4-methylbenzhydrylamine
Meb	*p*-methylbenzyl
Men	*β*-menthyl
MeSub	2-methoxy-5-dibenzosuberyl
Met	methionine
MIM	1-methyl-3-indolylmethyl
mio-Fmoc	2-monoisooctyl-Fmoc
MIS	1,2-dimethylindole-3-sulfonyl
Mmt	monomethoxytrityl
MNPPOC	2-(3,4-methylenedioxy-6-nitrophenyl)propyloxycarbonyl
Mob	*p*-methoxybenzyl
Mpe	*β*-3-methylpent-3-yl
Msc	2-(methylsulfonyl)ethoxycarbonyl
Mtr	4-methoxy-2,3,6-trimethylphenylsulfonyl
Mts	mesitylene-2-sulfonyl
Mtt	4-methyltrityl
NCA	*N*-carboxy anhydrides
Nin	ninhydrin
NMM	*N*-methyl mercaptoacetamide
NMP	1-methylpyrrolidin-2-one
NPPOC	2-(2-nitrophenyl)propyloxycarbonyl
Nps	2-nitrophenylsulfanyl
Npys	3-nitro-2-pyridinesulfenyl
Nsc	2-(4-nitrophenylsulfonyl)ethoxycarbonyl
α-Nsmoc	1,1-dioxonaphtho[1,2-*b*]thiophene-2-methyloxycarbonyl
NVOC	6-nitroveratryloxycarbonyl
*o*NBS	*o*-nitrobenzenesulfonyl
*o*NZ	*o*-nitrobenzyloxycarbonyl
Orn	ornithine
Pac	phenacyl
Pbf	pentamethyl-2,3-dihydrobenzofuran-5-sulfonyl
Pen	pentyl
PhAcm	phenylacetamidomethyl
Phdec	phenyldithioethyloxycarbonyl
2-Ph^iPr	2-phenylisopropyl
*p*HP	*p*-hydroxyphenacyl
Pmbf	2,2,4,6,7-pentamethyl-5-dihydrobenzofuranylmethyl
Pmc	2,2,5,7,8-pentamethylchroman-6-sulfonyl
Pms	2-[phenyl(methyl)sulfonio]ethyloxycarbonyl tetrafluoroborate
PNA	peptide nucleic acid
*p*NB	*p*-nitrobenzyl
*p*NBS	*p*-nitrobenzenesulfonyl
*p*NZ	*p*-nitrobenzyloxycarbonyl
Poc	propargyloxycarbonyl
ΨPro	pseudoprolines
Pydec	2-pyridyldithioethyloxycarbonyl
Ser	serine
SPPS	solid-phase peptide synthesis
Sps	2-(4-sulfophenylsulfonyl)ethoxycarbonyl
SPS	solid-phase synthesis
S-Pyr	2-pyridinesulfenyl
S^tBu	*tert*-butylmercapto
Sub	5-dibenzosuberyl
Suben	*ω*-5-dibenzosuberenyl
TAEA	tris(2-aminoethyl)amine
TBAF	tetrabutylammonium fluoride
TBDMS	*tert*-butyldimethylsilyl
TBDPS	*tert*-butyldiphenylsilyl
^tBu	*tert*-butyl
TCA	trichloroacetic acid
Tce	2,2,2-trichloroethyl
TCEP	*tris*-carboxymethylphosphine
TCP	tetrachlorophthaloyl
TEA	triethylamine
TEAF	tetraethylammonium fluoride
TFA	trifluoroacetic acid
tfa	trifluoroacetyl
TFE	2,2,2-trifluoroethanol
TFMSA	trifluoromethanesulfonic acid
Thr	threonine
Tmob	2,4,6-trimethoxybenzyl
TMS	trimethylsilyl
TMSE	trimethylsilylethyl
Tmsi	2-(trimethylsilyl)isopropyl
Tos	tosyl
Troc	2,2,2-trichloroethyloxycarbonyl
Trp	tryptophan
Trt	trityl
Tyr	tyrosine
Xan	9-xanthenyl
Z	benzyloxycarbonyl

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