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Orthogonal Protecting Groups for N^α -Amino and C-Terminal Carboxyl Functions in Solid-Phase Peptide Synthesis

Abstract: For the controlled synthesis of even the simplest dipeptide, the N^α -amino group of one of the amino acids and the C-terminal carboxyl group of the other should both be blocked with suitable protecting groups. Formation of the desired amide bond can now occur upon activation of the free carboxyl group. After coupling, peptide synthesis can be continued by removal of either of the two protecting groups and coupling with the free C-terminus or N^α -amino group of another protected amino acid. When three functional amino acids are present in the sequence, the side chain of these residues also has to be protected. It is important that there is a high degree of compatibility between the different types of protecting groups such that one type may be removed selectively in the presence of the others. At the end of the synthesis, the protecting groups must be removed to give the desired peptide. Thus, it is clear that the protection scheme adopted is of the utmost importance and makes the difference between success and failure in a given synthesis. Since R. B. Merrifield introduced the solid-phase strategy for the synthesis of peptides, this prerequisite has been readily accepted. This strategy is usually carried out using two main protection schemes: the *tert*-butoxycarbonyl/benzyl and the 9-fluorenylmethoxycarbonyl/*tert*-butyl methods. However, for the solid-phase preparation of complex or fragile peptides, as well as for the construction of libraries of peptides or small molecules using a combinatorial approach, a range of other protecting groups is also needed. This review summarizes other protecting groups for both the N^α -amino and C-terminal carboxyl functions. © 2000 John Wiley & Sons, Inc. Biopoly 55: 123–139, 2000

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INTRODUCTION

Peptide synthesis is based on the appropriate combination of protecting groups together with an efficient method for the activation of the carboxyl group prior to reaction with the amino component.¹ In the solid-phase strategy developed by Merrifield, the C-terminal protecting group is in fact a polymeric carrier and, consequently, the synthesis is carried out on an insoluble support.² The solid-phase method now domi-

nates synthetic peptide chemistry and the majority of peptides are made using this technique.

Since Merrifield described the solid-phase approach, only two *protection schemes* have been widely adopted. The *tert*-butoxycarbonyl (Boc)/benzyl (Bzl) strategy depends on graduated acid lability.² Thus, while the Boc group is removed by trifluoroacetic acid (TFA), Bzl, and related protecting groups are removed at the end of the synthetic strategy with strong acids such as HF or trifluoromethanesulfonic.

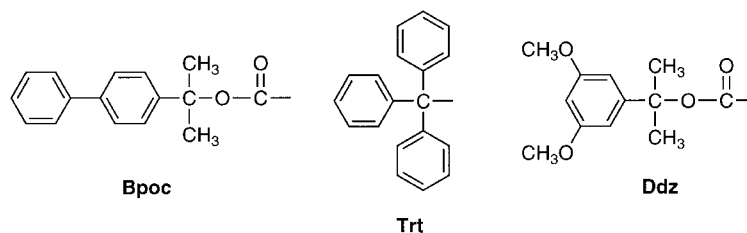


FIGURE 1 Structure of acid-labile protecting groups.

The main drawback associated with this strategy is a lack of flexibility. Long exposure of the peptide chain to TFA in the removal of the Boc group can also cause premature removal of the benzyl protecting group. Furthermore, conditions for the removal of Bzl groups will always remove the Boc group. Finally, some peptides containing fragile sequences will not survive the strong acid conditions used to remove the Bzl groups. The alternative 9-fluorenylmethoxycarbonyl (Fmoc)/*tert*-butyl (*t*-Bu) strategy^{3,4} is based on the *orthogonal* concept⁵ in the sense that the two protecting groups belong to independent classes and are removed by different mechanisms. The two groups can be removed therefore in any order in the presence of the group. Orthogonal protection schemes are milder because the selective deprotection is governed by alternative cleavage mechanisms rather than by reaction rates. The Fmoc group⁶ is removed by piperidine through a β -elimination reaction and *t*Bu is removed by acidolysis with TFA. Although this strategy has several advantages with respect to the Boc/Bzl approach, the conditions are still, in some cases, too harsh and can be incompatible with certain sequences. Furthermore, the preparation of complex molecules such as cyclic or branched systems could require the use of other protecting groups.⁷ In this report, other protecting groups for the amino function as well as for the carboxyl one are discussed.

***N* $^{\alpha}$ -AMINO PROTECTING GROUPS**

Acid-Labile Protecting Groups

Groups that can be removed through a milder acid in comparison to the Boc group are 2-(4-biphenyl)isopropoxycarbonyl (Bpoc),⁸ triphenylmethyl (trityl, Trt),⁹ and α,α -dimethyl-3,5-dimethoxybenzyloxycarbonyl (Ddz)¹⁰ (Figure 1).

The Bpoc group can be removed by treatment with 0.2–0.5% TFA* in CH_2Cl_2 and has been used in combination with *t*-Bu side-chain protecting groups in the early stage of solid-phase peptide synthesis (SPPS).^{11,12}

The main disadvantage of these derivatives is that most are obtained as oils that undergo an autocatalytic decomposition to the amino acid, CO_2 , and the Bpoc olefin and its dimer over a half-life of weeks.¹³ Thus, they have to be isolated and stored as cyclohexyl ammonium (CHA) or dicyclohexyl ammonium (DCHA) salts. Liberation of the free carboxyl compounds is not a straightforward process due to the extremely high acid lability of the protecting group. Recently, a convenient method for the preparation of the *O*-pentafluorophenyl (*O*-Pfp) esters has been reported.¹³ Most of these compounds are obtained as crystalline solids. Furthermore, the Pfp ester is intended both to protect the highly acid-labile urethane from autocatalytic cleavage by the free acid during storage and to activate the derivative for acylation during peptide coupling reactions. The preparation of the Pfp esters of Bpoc-amino acids was performed by first reacting the starting amino acid derivative with either Bpoc-phenyl carbonate, Bpoc-azide, or Bpoc-*p*-methoxycarbonylphenyl carbonate to form the free Bpoc-amino acids, which were immediately esterified with dicyclohexylcarbodiimide (DCC).

SPS of several model peptides has been performed using Pfp esters of Bpoc-amino acids (4 equiv) in the presence of 1-hydroxybenzotriazole (HOBt; 4 equiv), an additive to form in situ the OBt esters and therefore to increase the reactivity, and diisopropylethylamine (DIEA, 8 equiv) to neutralize Pfp-OH and HOBt, both of which are sufficiently acidic to present a threat to the stability of the Bpoc group, in CH_2Cl_2 /*N,N*-

* When oxymethyl-containing resins such as poly(ethylene glycol)-based resins are used, higher contents of TFA for this and other acid-labile protecting groups could be necessary because some of the TFA will be employed for the protonation of the oxymethyl moieties.

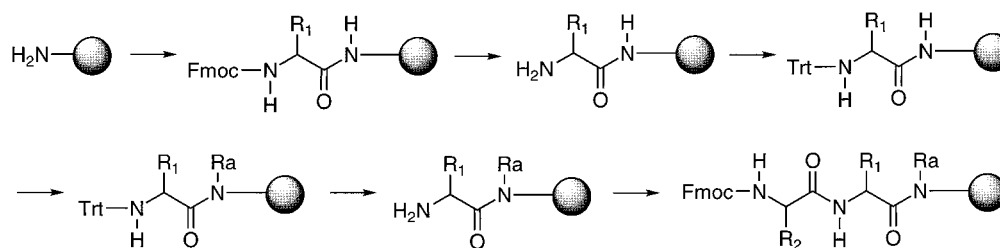


FIGURE 2 Scheme for the alkylation of amide bonds. Adapted from Ref. 22.

dimethylformamide (DMF) (1:1). Removal of the protecting group was achieved with 0.5% TFA in CH_2Cl_2 ($1 \times 3 \text{ min} + 1 \times 15 \text{ min}$). In this regard, the use of scavengers such as benzyl mercaptan and thiophenol has been also reported.¹⁴

The Trt group can be removed in solution with 1% TFA in CH_2Cl_2 ¹⁵ and 0.1M HOBt in trifluoroethanol (TFE).¹⁶ To extend the application of Trt-amino acids in solid-phase synthesis, as well as their compatibility with hyperacid-labile resins and acid-sensitive biomolecules, the use of mild acid conditions was investigated.¹⁷ The results indicate that 0.1M 7-aza-1-hydroxybenzotriazole (HOAt)/0.12M Me_3SiCl in TFE, 0.25M Me_3SiCl in TFE, 0.2% TFA/1% H_2O in CH_2Cl_2 , and 3% trichloroacetic acid (TCA) in CH_2Cl_2 all quantitatively remove the Trt group and are compatible with the 3-(4-hydroxymethylphenoxy)propionic acid (AB) linker. Furthermore, solutions of 0.2% TFA/1% H_2O in CH_2Cl_2 , and 3% TCA in CH_2Cl_2 are compatible with the most labile Riniker handle and the synthesis of oligonucleotide-peptide conjugates, respectively.

The synthesis of Trt-amino acids can be carried out starting either from the amino acid or the methyl ester.^{9,18} The former method involves in situ trimethylsilyl ester formation to protect the carboxylic acid followed by reaction with Trt-Cl. The latter method requires reaction with Trt-Cl followed by hydrolysis of the methyl ester with $\text{LiOH}/\text{H}_2\text{O}/\text{CH}_3\text{OH}$ at 25–40°C. In our laboratories the second strategy is preferred since the overall yields are better (>75% as opposed to 40–60%) and column chromatography is not required to obtain pure derivatives.¹⁹

The main drawback associated with Trt-amino acids is that these compounds couple with other amino acid derivatives in lower yields when compared with carbamate-based protected amino acids.⁹ SPPS with Trt-amino acids have proceeded in good yields when *N*-[(dimethylamino)-1*H*-1,2,3-triazolo[4,5-*b*]pyridino-1-ylmethylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide (HATU) in the presence of DIEA was used as the coupling reagent. Thus, several model peptides have been synthesized using

single couplings with HATU/DIEA (4 equiv:8 equiv) for 20 min and with 3% TCA in CH_2Cl_2 ($2 \times 3 \text{ min}$) for removal of the Trt group.¹⁹

The Trt group could be used in combination with an Fmoc strategy in order to avoid diketopiperazine (DKP) formation in a similar manner to that described earlier for a Boc strategy.²⁰ This procedure avoids the presence of the free amino function of the penultimate residue and incorporates the third residue with neutralization in situ. Thus, in those sequences that are prone to DKP formation, the following experimental protocol should be used: (i) incorporation of the penultimate residue as its Trt derivative, (ii') selective detritylation with TFA/ $\text{H}_2\text{O}/\text{CH}_2\text{Cl}_2$ (2:1:97) ($5 \times 1 \text{ min}$) for AB-type resins or (ii'') TFA/ $\text{H}_2\text{O}/\text{CH}_2\text{Cl}_2$ (0.2:1:99; $5 \times 1 \text{ min}$) for Riniker-based resin, and (iii) incorporation of the third residue as its Fmoc derivative under in situ neutralization/coupling conditions mediated by 7-azabenzotriazol-1-yl-*N*-oxy-tris(pyrrolidino)phosphonium hexafluorophosphate (PyAOP)/DIEA (5 equiv each) in DMF for 1 h.²¹

The Trt protecting group has also been used in a strategy developed to successively alkylate each amide bond following its formation.²² Thus, after the coupling of an Fmoc-amino acid, the Fmoc group is removed and the *N*-terminal amino function is reprotected with Trt by reaction with Trt-Cl in the presence of DIEA. Treatment of the Trt-peptide resin with lithium *t*-butoxide in tetrahydrofuran (THF) leads to the formation of the amide anion. Following removal of excess base, the alkylating reagent in DMSO is reacted with the Trt-peptide resin. The alkylation reaction mixture is then removed and the base and alkylation treatments repeated to drive the alkylation to completion. Removal of the Trt group, followed by coupling of the next Fmoc-amino acid allows the formation of the next amide bond, which can again be alkylated by following the same protocol involving the concurrence of Trt group (Figure 2).

The Ddz-amino acids, which are the most commercially available, are more stable in the presence of acids than both the Bpoc and the Trt analogues. The

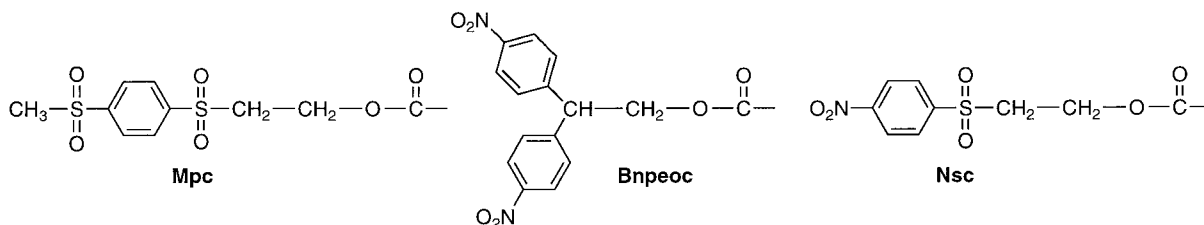


FIGURE 3 Structure of protecting groups that are base labile through a β -elimination reaction.

Ddz group can be removed with 1–5% TFA in CH_2Cl_2 and the protected amino acids are described as being fully compatible with *t*-Bu side-chain protecting groups.²³ Furthermore, Ddz is also removed by photolysis at wavelengths above 280 nm. This property also makes the Ddz group potentially very useful in SPS library screening procedures. Ddz derivatives have a very characteristic uv absorption pattern with maxima at 230, 276, and 282 nm. Therefore, the Ddz fission product allows a very precise determination of the initial loading of the first amino acid on the resin as well as of the growing Ddz-peptide resin.

Ddz-amino acids have also been used to avoid DKP formation when a Backbone Amide Linker (BAL) strategy is used.²⁴ As mentioned above for AB- and Riniker-type resins, the incorporation of the penultimate residue is performed using the Ddz derivative. Removal of the protecting group is achieved with $\text{TFA-H}_2\text{O-CH}_2\text{Cl}_2$ (3:1:96) for 6 min before incorporation of the third residue as its Fmoc derivative under in situ neutralization/coupling conditions mediated by PyAOP. The advantage of the Ddz protecting group over the Trt group is that the former couples more efficiently, which is an especially important factor when the penultimate residue has to be incorporated to a second and/or hindered amine.²⁴

Base-Labile Protecting Groups

Although the use of Fmoc-based SPPS has increased enormously in recent years to the point where it is now probably the method of choice for the chemical synthesis of peptides, there could also be a need to search for other, base-labile amino protecting groups. The main drawbacks associated with the use of Fmoc-amino acids are related to their hydrophobicity. For example, some derivatives possess low solubility in the solvents commonly used in SPPS, which can cause low coupling yields and/or problems associated with automation. Solubility issues are more critical during the manipulation of Fmoc-protected peptides in a convergent strategy.^{25,26}

During the last few years, several N^α -amino protecting groups have been reported to overcome some of the problems described above. Examples of these are 2-[4-(methylsulfonyl)phenylsulfonyl]ethoxycarbonyl (Mpc),²⁷ 2,2-bis-(4'-nitrophenyl)ethoxycarbonyl (Bnpeoc),²⁸ and 2-(2,4-dinitrophenyl)ethoxycarbonyl (Dnpeoc)²⁹ (See Figure 3).

Particularly appealing is the 2-(4-nitrophenylsulfonyl)ethoxycarbonyl (Nsc) group,^{27,30,31} which can be considered from the point of chemical composition to be a combination of the Dnpeoc and the Mpc groups, since both nitro and sulfonyl groups are incorporated into the structure. These two functional groups should enhance the solubility of an amino acid and prevent hydrophobic interactions between the peptide chains, a situation that should subsequently reduce the degree of failure or truncated sequences during chain elongation.^{32,33} Nsc-amino acids are synthesized easily from the corresponding succinimidyl carbonate and are crystalline compounds, a physical property strongly preferred for automated SPPS. Furthermore, the mechanism for the removal of the Nsc group is similar to that for Fmoc deprotection and is based upon a base-catalyzed β -elimination reaction. Therefore, the chemistry and instrumentation described for the Fmoc strategy can be readily adapted to this protecting group.

The removal of the Nsc group proceeds at a rate three to ten times slower than the removal of the Fmoc group, and is dependent on the nature of the base and solvent (Table I).³⁴ The high stability of Nsc in DMF is of particular importance when this group is to be left on for long period of time. Thus, the Nsc group is more suitable for use in automatic synthesizers where amino acid derivatives are stored in solution for relatively long periods or when protected peptides are left to react in a convergent strategy. In this sense, when traces of piperidine remain, the removal of Fmoc is still much faster.³⁴ Nsc is clearly a better protecting group as far as stability is concerned. Conditions recommended for the removal of Nsc are 20% piperidine in DMF, or preferably DMF–dioxane (1:1), for 15 min. The addition of the stronger base

Table I Comparison Between Fmoc and Nsc Protected Peptides^a

	Fmoc	Nsc
Cleavage rate ($t_{1/2}$)		
20% Piperidine/DMF	10–15 s	90–110 s
1% DBU/20% piperidine/DMF	—	12–15 s
Decomposition in DMF solution		
1 week	10%	< 1%
3 weeks	40%	2%
Olefin-amine adduct formation	Fast and reversible	Very fast and irreversible
Polymerization during removal	Yes	No
uv monitoring range	302 nm	380 nm

^a Source: Ref. 34.

1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) can compensate for the slower rate of N^α -deprotection.³⁰ In this case, caution is required because the use of DBU, even at a concentration of 1% (v/v), can cause aspartimide formation in sensitive sequences.³⁵ Other advantages that Nsc has over the Fmoc group are that the formation of the olefin-amine adduct is irreversible and takes place faster than with Fmoc.³⁴ The Nsc group absorbs at 380 nm, which allows selective online monitoring during SPPS.

Coupling kinetic studies have indicated that while Fmoc-amino acids at times coupled slightly faster than Nsc derivatives, both were globally within the same range of reactivity.³⁶

Premature removal of the N^α -Fmoc protecting group has been observed during the synthesis of polyproline-containing peptides.³⁷ This phenomenon is presumably due to the secondary amine character of the α -amino function of the Pro. Electrospray mass spectroscopy analysis of the sequence H-PPPPPPA-NH₂ prepared with Nsc-Pro-OH showed no evidence of Pro insertion (detected as an [M + 97] peak), which would be indicative of premature deprotection of a Pro and the subsequent coupling of another protected Pro.³⁶

The loss of configuration at the C-terminal carboxyl residue is perhaps the most important side reaction in peptide synthesis.³⁸ This is often enhanced by the use of highly potent coupling reagents, which usually convert the carboxyl function to a derivative bearing a good leaving group. Such leaving groups tend to increase the acidity of the α -proton and favor oxazolone formation, both of which lead to racemization.³⁹ This risk is more accentuated when residues such as His,⁴⁰ Cys,^{41,42} and Ser⁴³ are incorporated into a sequence. To compare the racemization potential of Nsc- and Fmoc-amino acids, the model tripeptides H-Gly-Xxx-Phe-NH₂ (where Xxx = His, Cys, and Ser)^{42,43} were manually synthesized by each method in the solid phase.³⁶ Preactivation of the carboxyl group was avoided to minimize racemization of the susceptible residues. High performance liquid chromatography (HPLC) analysis (Table II) revealed that for the Nsc-amino acids, racemization was undetected for Ser and reduced by more than a half for Cys and His. This illustrates the value of N^α -Nsc Cys and His residues since Fmoc-amino acids gave unacceptable results. Presumably, the higher stability of the Nsc-derivatives is correlated with the reduced acidity of the β -proton of the protecting group, which trans-

Table II Racemization Studies^a

H-Gly-aa-L-Phe-NH ₂	aa: His		aa: Cys		aa: Ser	
	% L,L	% D,L	% L,L	% D,L	% L,L	% D,L
Fmoc-L-aa-OH	97.5	2.5	89.9	10.1	98.9	1.1
Fmoc-D-aa-OH	2.1	97.9	4.9	95.1	1.0	99.0
Nsc-L-aa-OH	98.9	1.1	95.7	4.3	99.9	0.1

^a Source: Ref. 36.

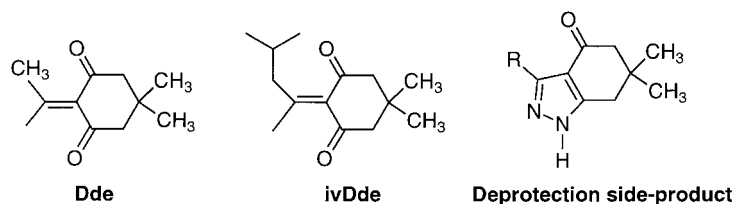


FIGURE 4 Structure of Dde and ivDde protecting groups and the indazole derivative formed during the deprotection step.

lates to a lower acidity of the α -proton of the amino acid. However, dedicated protocols for the automatic synthesis of peptides with a preactivation step using either Nsc or Fmoc chemistry should be developed. The use of weaker bases such as collidine (pKa 7.43) instead of DIEA (pKa 10.1) is a potential strategy as illustrated by studies performed on model systems.^{42–45}

In conclusion, the Nsc group can be considered an alternative to Fmoc for N^α -protection.^{34,36,46} Nsc-amino acids are more suitable for use in automatic synthesizers where amino acid derivatives are stored in solution over relatively long periods or when protected peptides are left to react in a convergent strategy. In this sense, the presence of an Nsc moiety, due to its polarity, should also facilitate the purification of protected peptides. Furthermore, the use of Nsc derivatives of Cys and His reduces the racemization of these residues when compared with the corresponding Fmoc derivatives. The preparation of proline-rich peptides occurs without premature removal of the Nsc group.

Although the 1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl (Dde)⁴⁷ group can be used for the N^α -protection, it has been mainly used for the protection of the ϵ -amino function of Lys/Orn.^{48,49} Deprotection, which results in the formation of 3,6,6-trimethyl-4-oxo-4,5,6,7-tetrahydro-1H-indazole, is carried out by hydrazine–DMF (2:98) for 5 min, and can be monitored by the absorption at 300 nm. Dde is almost completely stable to conditions used to remove the Fmoc group. However, this small loss of Dde can compromise the purity of large peptides. Furthermore, Dde has also been reported to undergo intramolecular $N \rightarrow N'$ migration from side-chain or α -amino group to the ω -function of Lys/Orn,⁵⁰ resulting in the scrambling of the group within the peptide chain. In order to avoid both side reactions, the 1-(4,4-dimethyl-2,6-dioxocyclohexylidene)-3-methylbutyl (ivDde)⁵¹ group has been described. This is removed in the same conditions than Dde. (See Figure 4.)

Recently, a new family of N^α -amino protecting groups, whose removal is achieved by nucleophilic addition to a Michael acceptor, has been de-

scribed.^{52–54} The key deprotection step in the case of amines protected by either the 2-*tert*-butylsulfonyl-2-propenoxycarbonyl (Bspoc),⁵³ the 1,1-dioxobenzo-[b]thiophene-2-ylmethyloxycarbonyl (Bsmoc),⁵² or the 2-methylsulfonyl-3-phenyl-1-prop-2-enyloxycarbonyl (Mspoc)⁵⁴ groups involves the addition of a nucleophilic reagent to the α,β -unsaturated sulfone system with the consequent ejection of the carbamate ion. (See Figures 5 and 6) There are several major advantages of such a process over the classic β -elimination process involved in the removal of groups such as Fmoc or Nsc: (i) the deblocking event is simultaneously a scavenging event, thus avoiding back alkylation of the β -elimination by-product, e.g., dibenzofulvene⁵²; (ii) lower concentrations of secondary amine (piperidine or morpholine) can be used, thus minimizing base-catalyzed side reactions such as aspartimide formation^{52,55}; and (iii) the method can be applied to the technique of rapid continuous solution synthesis.⁵⁵ In turn, the Mspoc group has some advantages with respect to the other two approaches in question. It is less sensitive to premature deblocking than the Bspoc and can be assembled from readily available materials without recourse to low-valent sulfur intermediates, as required in the case of the Bsmoc.⁵⁴

Due to the different lability in the presence of nucleophiles and the difference in the removal mechanism, fluorenylmethyl (Fm)- and 1,1-dioxobenzo[b]thiophene-2-ylmethyl (Bsm)-based protecting groups can be orthogonally removed by the appropriate choice of deblocking reagent.^{52,55} Thus, Bsmoc can be removed in the presence of an Fm ester by treatment with 2% tris(2-aminoethyl)amine (TAEA)

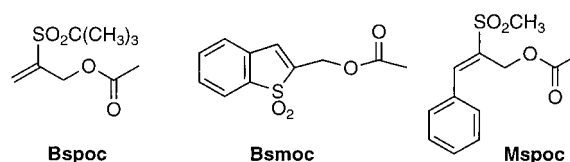


FIGURE 5 Structure of protecting groups that are base labile through a Michael addition.

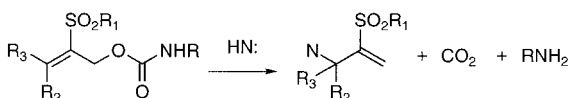


FIGURE 6 Mechanism for the removal of protecting groups that are base labile through a Michael addition. Adapted from Ref. 22.

and Fmoc can be removed in the presence of a Bsm ester by *N*-methyl-*tert*-butylamine, the steric hindrance of which avoids extensive Michael-like addition.

Several model peptides have been synthesized in solid-phase using Bsmoc-amino acids.⁵² The removal of the Bsmoc group was accomplished with 2–5% piperidine in DMF. The use of 2% piperidine in DMF for 7 min leads to a reduction in the extent of aspartimide formation, e.g., 4.8 vs 11.6% obtained when 20% piperidine is used for the removal of Fmoc group in a sequence prone to give this side reaction. An additional advantage of Bsmoc over Fmoc assembly was noted in the case of pentapeptide H-Tyr-Aib-Aib-Phe-Leu-OH, where the difficult Aib-Aib coupling was favored by about 13%.

Pd-Labile Protecting Groups

In 1950 the allyloxycarbonyl (Alloc) group was introduced by Stevens and Watanabe for the protection of amine and alcohol functions.⁵⁶ They reported that cleavage of the Alloc group was accomplished by catalytic hydrogenolysis using platinum or palladium catalysts, the use of metallic sodium in liquid ammonia, or the use of phosphonium iodide in glacial acetic acid (HOAc). Unfortunately, these methods for the removal of the Alloc group were not straightforward, which limited its use in organic synthesis. With the palladium-catalyzed allyl removal reaction developed by Tsuji⁵⁷ and Trost,⁵⁸ the potential of the allyl protecting group was recognized.^{59,60} The deprotection step involves a palladium-catalyzed transfer of the allyl unit to various nucleophiles/scavengers in the presence of a proton source (Figure 7). Thus, allyl-based groups are orthogonal with both base-labile, such as Fm, and acid-labile, such as *t*-Bu, protecting groups.

Allyl chemistry has mainly been used in solid-phase strategies either as a handle or as a side-chain protecting strategy. Thus, Kunz and co-workers⁶¹ first introduced the use of 4-bromocrotonic acid coupled to aminomethyl polystyrene resin as an allylic anchor. Since then, other handles have also been used for the preparation of a broad range of peptides.^{25,60,62} Hudson and co-workers⁶³ and Loffet and Zhang⁶⁴ independently investigated the use of the allyl group as a side-chain protecting group. The three-dimensional orthogonal strategy (Fmoc/*t*-Bu/allyl) has been mainly used for the preparation of cyclic peptides.^{65,66}

The *N*^α-Alloc-amino acids are synthesized easily from the corresponding chloroformates⁶⁷ using the method developed by Bolin and co-workers for the corresponding Fmoc derivatives.⁶⁸ It should be mentioned that even for hindered amino acids such as Val the formation of the protected dipeptide has been observed.⁶⁹ This method involves the *in situ* preparation of the trimethylsilyl derivatives and subsequent reaction with the chloroformate. Although these derivatives are obtained as oils, they can be crystallized, purified, and stored as their dicyclohexylammonium salts. Alternatively, the pentafluorophenyl esters, which are crystalline, can be easily prepared using the procedure described by Green and Berman for the Fmoc derivatives.⁷⁰ These active esters represent an excellent method of storage for *N*^α-Alloc amino acids and as active species can be used directly for synthetic purposes.⁶⁷ In this case, it is convenient to perform the coupling in the presence of an equivalent of HOBT or HOAt in order to accelerate the process.

The main concern with the use of the Alloc group for *N*^α-amino protection is the formation of allylamines. The consequences of this side reaction can be more important when the Alloc group is used in a repetitive way for temporary *N*^α-amino protection in SPPS. Allylamine formation can take place during the Pd-catalyzed removal of allylcarbamates and may occur in different ways.⁶⁰

First of all, the free amine may compete with the nucleophilic allyl-group scavenger in the trapping of the π -allyl palladium complex. Therefore, it is better to use deprotection systems that lead to the protonated

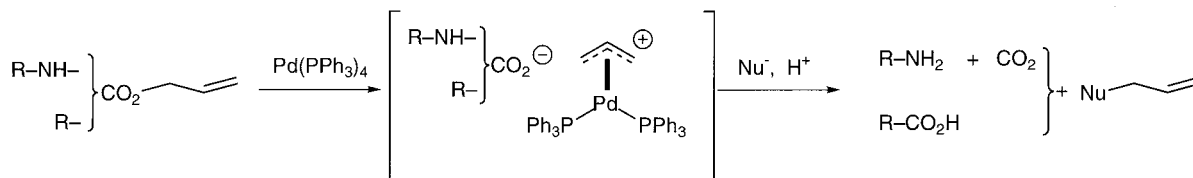


FIGURE 7 Palladium-catalyzed deprotection of allyl carbamates and carboxylates.

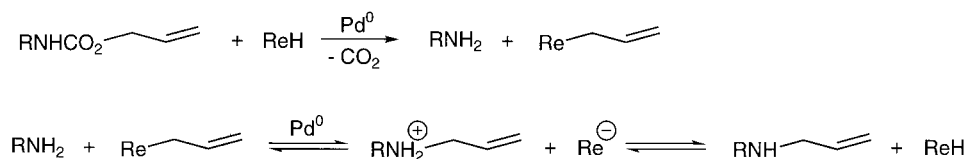


FIGURE 8 Mechanism of allylamine formation when protonic reversible allyl-group scavengers are used. Adapted from Ref. 60.

amine or other masked non-nucleophilic forms of the amine, or to use the allyl scavenger in large excess. With the protonic reversible allyl-group scavengers ReH, allylamine may also be formed through an equilibration process (Figure 8). Finally, Allocamines are known to undergo decarboxylative rearrangement to allyl amines in the presence of Pd catalysts and in the absence of nucleophiles.⁷¹

The choice of an appropriate allyl scavenger is clearly a key factor for the establishment of a convenient strategy for the use of *N*^α-Alloc as a temporary protecting group in SPPS. Guibé and co-workers⁷² described the first SPPS using this protecting group. In this work, the removal of the Alloc group was effected by the hydrostannolytic procedure, which utilizes tributyltin hydride as the allyl group scavenger. This procedure was selected on the grounds that the hydrostannolytic procedure is extremely fast, being complete within a few minutes (thus minimizing the risk of allylamine formation), and very selective, since tributyltin hydride is nonbasic and is, at room temperature, inert toward almost any reducible functions that do not interact with soluble palladium catalysts. On using this method the peptide was obtained in a moderate, nonoptimized yield of 25%. Two draw-

backs, however, are associated with the use of tributyltin hydride. The first is that tributyltin hydride is not easy to handle and, in particular, shows a propensity, in the presence of various agents including palladium catalysts, to decompose into hexabutyldistannane and dihydrogen.⁷² The second drawback is associated with the toxicity of tin compounds and the difficulty often encountered in their complete removal from the desired end products (this last difficulty is minimized in the case of synthesis on solid supports). Other allyl group scavengers have been investigated with the aim of developing alternative tin-free methods that are well suited for repetitive Alloc removal and compatible with the presence of Fmoc and *t*-Bu protecting groups.⁶⁷ Table III shows the results of a study carried out in which the Alloc derivative of the *N*-methylbenzylamine was taken as a model and shows a higher propensity to form allylamine than Alloc derivatives of primary amines. The study includes the pronucleophilic species (NDMBA) and thiosalicylic acid (TSA) proposed by Kunz⁷³ and Genêt,⁷⁴ respectively, and pseudometallic hydrides, i.e., borohydrides (BH₄⁻), initially proposed by Zhu and co-workers,⁷⁵ phenylsilane (PhSiH₃) proposed by Guibé and co-workers,⁷⁶ and several as yet untested

Table III Palladium-Catalyzed Deprotection [Pd(Ph₃)₄] of the Alloc Derivative of *N*-Methylbenzylamine in the Presence of Various Allyl Group Scavengers^a

Allyl Group Scavenger	Yield (%)	
	H—N(Me)CH ₂ Ph	All—N(Me)CH ₂ Ph
NDMBA	100	0
TSA	100	0
PhSiH ₃	95	5
Bu ₄ N ⁺ BH ₄ ⁻	60	40
NH ₃ · BH ₃	99.6 to 100	0 to 0.4
(Me) ₂ NH · BH ₃	100	0
<i>t</i> -BuNH ₂ · BH ₃	96 to 97	3 to 4
(Me) ₃ N · BH ₃	0	100
Py · BH ₃	0	100

^a Source: Ref. 67.

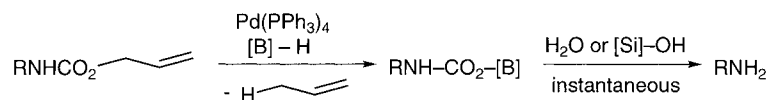


FIGURE 9 Palladium-catalyzed deprotection of allyl carbamates in the presence of boranes. Adapted from Ref. 67.

amine-borane complexes including $\text{NH}_3 \cdot \text{BH}_3$, $(\text{Me})_2\text{NH} \cdot \text{BH}_3$, $t\text{-BuNH}_2 \cdot \text{BH}_3$, $(\text{Me})_3\text{NH} \cdot \text{BH}_3$, and $\text{Py} \cdot \text{BH}_3$.

The pronucleophilic scavengers NDMBA and TSA, and the amine borane complexes $\text{H}_3\text{N} \cdot \text{BH}_3$ and $\text{Me}_2\text{NH} \cdot \text{BH}_3$, were found to lead selectively to *N*-methylbenzylamine. Meanwhile, concurrent and increasing formation of *N*-allyl- and *N*-methylbenzylamine was observed in the series $t\text{-BuNH}_2 \cdot \text{BH}_3 \leq \text{PhSiH}_3 < \text{BH}_4^-$. As far as the experiments with $\text{Py} \cdot \text{BH}_3$ and $\text{Me}_3\text{N} \cdot \text{BH}_3$ are concerned, these reactions led exclusively to the *N*-allylated amine. Clearly the tertiary amine-borane complexes do not act as allyl group scavengers at all. From the above experiments and with regard to selectivity, the pronucleophilic species NDMBA and TSA, on the one hand, and the amine-borane complexes $\text{H}_3\text{N} \cdot \text{BH}_3$ and $\text{Me}_2\text{NH} \cdot \text{BH}_3$, on the other hand, appear to be the best-suited scavengers for the palladium-catalyzed removal of *N*-Alloc groups. With regard to the reactivity, however, the amine-borane complexes that lead to complete deprotection in less than 10 min are far superior to the pronucleophilic species, for which reaction times of between 30 and 90 min are required for the reaction to reach completion. Therefore, only the two amine-borane complexes $\text{H}_3\text{N} \cdot \text{BH}_3$ and $\text{Me}_2\text{NH} \cdot \text{BH}_3$ can be recommended as deprotecting agents for use in SPPS strategies. It should be noted that, in a similar way to that observed with other pseudometallic hydrides such as tributyltin hydride⁷² or phenylsilane,^{76,77} the deprotection of *N*-Alloc derivatives with amine-borane complexes does not lead to the free amines but to the corresponding boron carbamates, which are readily hydrolyzed and decarboxylated upon exposure, for instance, to small amounts of water or silica. (See Figure 9.)

The stability of the Fmoc group and the ease of reduction of the Trp residue were assessed against these amine-borane complexes. It was found that Ac-Trp-OMe and Fmoc-Trp(Boc)-OMe were left unchanged after exposure at 25°C for 48 h to $\text{H}_3\text{N} \cdot \text{BH}_3$ and $\text{Me}_2\text{NH} \cdot \text{BH}_3$ either in the absence or the presence of $\text{Pd}(\text{Ph}_3)_4$. On the other hand, even in the absence of catalyst, Boc-Trp(CHO)-OMe is slowly reduced by $\text{H}_3\text{N} \cdot \text{BH}_3$ to the corresponding *N*-hy-

droxymethylindolyl derivative. The presence of $\text{Pd}(\text{Ph}_3)_4$ accelerates the reaction.

Thus, amine-borane complexes seem very attractive as allyl scavengers for a number of reasons. They are nonbasic and are in fact stable even in relatively strong acidic media. In addition, as reducing agents they are quite inert at room temperature toward functional groups usually encountered in peptide chemistry. At the same time, their hydride-donor ability toward palladium π -allyl complexes is very high, and finally, the expected ultimate by-products of the reaction (volatile amines and boric acid) should not interfere significantly in the purification steps, which should prove very convenient when the process is carried out in solution.

SPS of several model peptides has been carried out using Pfp esters of Alloc-amino acids (4 equiv) in DMF in the presence of HOBt (4 equiv), an additive to form in situ the OBt esters and therefore to increase the reactivity. Removal of the protecting group was achieved under an Ar atmosphere in CH_2Cl_2 at 25°C with 10 mol % (based on the substitution of the resin) of $\text{Pd}(\text{PPh}_3)_4$ and 6 equiv of $\text{H}_3\text{N} \cdot \text{BH}_3$ or $\text{Me}_2\text{NH} \cdot \text{BH}_3$. The amine-borane complex was *first* added to the resin with agitation, followed a few minutes later by the catalyst. After 10 min, the Pd solution was drained, the resin was washed with CH_2Cl_2 (3×30 s) and the deprotection procedure was repeated once more. The resin was then washed successively with CH_2Cl_2 (8×30 s), 0.2% TFA in CH_2Cl_2 (2×60 s), CH_2Cl_2 (5×30 s), DIEA- CH_2Cl_2 (1:19; 3×60 s) and CH_2Cl_2 (5×30 s).

PhSiH_3 also has been used as a scavenger in the SPS of a putative heptapeptide intermediate in the vancomycin biosynthesis⁷⁸ and also for Leu-enkephalin.⁷⁹ In this case, minor impurities <2% due to the corresponding allylpeptides were detected.

If the catalytic deallylation reaction is carried out in the presence of an acylating species, then a neat transacylation process takes place with the concomitant formation of the amide bond.⁷⁷ (See Figure 10.)

This method has also been used in the solid phase to avoid the formation of DKP.⁷⁹ The strategy involves the use of the Alloc group for the protection of the second residue in conjunction with the tandem

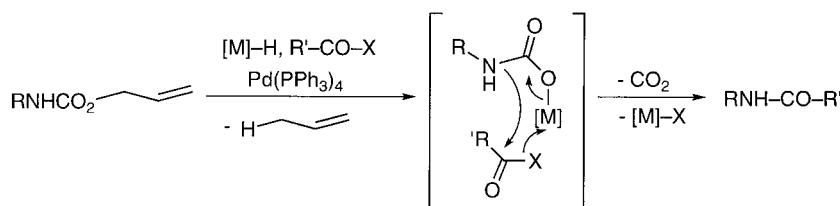


FIGURE 10 Tandem deprotection coupling of N^{α} -alloc-amino acids. Adapted from Ref. 77.

deprotection-coupling reaction. Formation of the new amide bond leading to the tripeptide resin under neutral conditions is more facile than DKP formation. Following the construction of the Alloc-dipeptide resin by a standard coupling protocol, the tandem deprotection-coupling reaction was carried out with $\text{Pd}(\text{PPh}_3)_4$ (0.10 equiv), PhSiH_3 (6 equiv), and the fluoride of the Fmoc-amino acid (5 equiv) in CH_2Cl_2 . Analysis of the HPLC and amino acid analysis (AAA) data of the peptide resin prior to cleavage and also of the corresponding peptide following its release from the support confirmed that DKP formation did not occur. Similar experiments were carried out in which the N -carboxyanhydride (NCA) derivative was replaced by fluoride and an equimolar mixture of Fmoc-amino acid, PyAOP, and DIEA was used. In the former case, DKP formation was observed at 2% whereas in the latter case DKP was not detected by AAA but the coupling of the third residue occurred in only 37% yield.

The removal of the Alloc group with $\text{Pd}(\text{PPh}_3)_4$ in the presence of, preferably, $\text{H}_3\text{N} \cdot \text{BH}_3$ and $\text{Me}_2\text{NH} \cdot \text{BH}_3$, or PhSiH_3 , makes this protecting group completely orthogonal to the presence of Fm and t -Bu-based groups. The use of other scavengers in an orthogonal scheme should be carefully evaluated before use. For example, the morpholine used by Kunz and Waldmann⁸⁰ can provoke removal of the Fmoc group, or the use of a solvent mixture of THF/DMSO/0.5M HCl (2:2:1), which has been shown to be very effective for the cleavage of peptides from allyl base resins,^{81,82} provokes the oxidation of the Met residue.⁸³

Finally, a convenient method has been described that allows the removal of the Alloc group in an automatic mode. In this case the use of $\text{CHCl}_3/\text{AcOH}$

N -methylmorpholine (NMM) (37:2:1) allows the total solubilization of $\text{Pd}(\text{PPh}_3)_4$ and is therefore compatible with most automatic synthesizers.^{83,84} Once the deprotection has taken place, the resin is washed with 0.5% diethyldithiocarbamate and 0.5% DIEA in DMF to remove particles of the catalyst. This method has been used for the automatic synthesis of cyclic and branched peptides, as well as for the stepwise synthesis of short peptides using N^{α} -Alloc-amino acids.

ARYLSULFONAMIDES

The recent introduction of o - and p -nitrobenzenesulfonyl (o - and p NBS) as protecting groups for amines by Fukuyama and co-workers⁸⁵ has generated much interest in this kind of derivative. N -Arylsulfonamides offer a number of interesting features that make them very appealing as temporary protecting groups. For example, the highly reactive N -arylsulfonylaminoacyl chloride can be used as the active species.⁸⁶ This contrasts with the Fmoc-amino acid chlorides, which in the presence of a hydrogen chloride acceptor such as DIEA or NMM give the corresponding oxazolone, which, although also a coupling reagent, is less reactive than the chloride.³⁹ The use of the chloride derivatives of arylsulfonamide protected amino acids allows the preparation of hindered peptides.^{87,88} Furthermore, the greater acidity of the ArSO_2NH subunit allows *site-specific alkylation* in the presence of an amide NH ,⁸⁸⁻⁹³ making these groups unique for the preparation of N -alkyl peptides. In addition, one interesting property of sulfonamides is that the derivatives are more crystalline than carbamates.⁹⁴ (See Figure 11.)

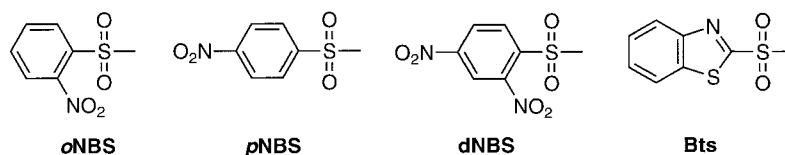


FIGURE 11 Structure of arylsulfonamide protecting groups.

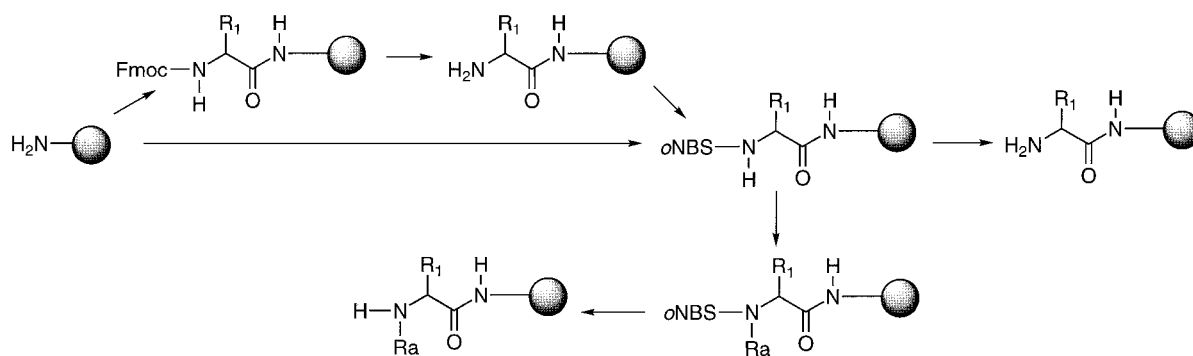


FIGURE 12 SPPS with oNBS protection. Adapted from Ref. 95.

The *o*-NBS-amino acids, most of which are solids, can be easily prepared from the commercially available and cheap 2-nitrobenzenesulfonyl chloride using the Schotten–Baumann procedure.⁹⁵ This approach has been rapidly applied in a solid-phase strategy for site-selective *N*-methylation of peptides on solid supports, as well as in conjunction with *t*-Bu side-chain protecting groups for the solid-phase preparation of free peptides.^{89,95} *o*NBS-amino acid chlorides were used to couple to extremely hindered amines on a solid support where the analogous Fmoc-amino acid chlorides failed to couple well.⁸⁹ The inability of *o*NBS-amino acids to form oxazolones suggests that these derivatives are less prone to racemization, as the primary mechanism of racemization for most amino acids proceeds via an oxazolone intermediate.^{39,95} For the acylation of unhindered residues, *o*NBS-amino acids couple perfectly in the presence of aminium salts, such as *N*-[(1*H*-benzotriazol-1-yl)(dimethylamino)methylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide (HBTU), in the presence of a base.⁸⁹ The removal of *o*NBS can be achieved with thiols through an *ipso* attack of the thio nucleophile to form an initial Meisenheimer-type complex, which, after loss of sulfur dioxide, gives a free amine (Path A, Figure 13).⁹⁶ Furthermore, the removal of *o*NBS from protected peptide resins releases a yellow chromophore [2-(2-nitrophenylthio)ethanol] that allows simple visual confirmation of deprotection.⁸⁹

After the incorporation of the *o*NBS-amino acid or the reprotection of the resin-bound peptide free amine with *o*NBS-Cl in CH₂Cl₂ in the presence of collidine, selective deprotonation of the sulfonamide with the strong, nonionic base 7-methyl-1,5,7-triazabicyclo-[4,4,0]dec-5-ene (MTBD) followed by reaction with methyl *p*-nitrobenzenesulfonate in DMF, led to the *N*-methyl peptide.⁸⁹ Alternatively, selective *N*-allylation can be performed using Pd(0) chemistry (allyl methyl carbonate in the presence of Pd₂dba₃/CHCl₃

and PPh₃ in THF).⁹⁵ Removal of the *o*NBS group from *N*-alkylated derivatives can be achieved with 2-mercaptoethanol and DBU, but these conditions are not applicable to nonalkylated *o*NBS derivatives, where the cleavage is much slower.⁸⁹ In this case, a 5% solution of thiophenol in DMF containing 2 equiv of K₂CO₃ relative to the thiol led to complete *o*NBS cleavage in less than 10 min. (See Figure 12.) For the synthesis of short model peptides, the use of *o*NBS- and Fmoc-amino acids led to comparable results.⁹⁵

The cleavage of the *para* regioisomer (*p*NBS) using thiolate has been found to give poor regioselectivity. In this case some addition occurs at the nitro carbon, resulting in simple displacement of the nitro group rather than sulfonamide cleavage. This lack of regioselectivity has not been observed for the *ortho* isomer.⁹⁷

The 2,4-dinitrobenzenesulfonyl (dNBS) protecting group has been removed by treatment with HSCH₂CO₂H and TEA.⁹⁸ These conditions are compatible with the presence of the mononitro derivative *o*NBS, thus allowing the preparation of a combinatorial library of diamine derivatives. Tomkinson et al. also used the 2,4-dinitrobenzenesulfonyl protecting group in solution in a novel deprotection/functionalization sequence for the one-pot preparation of amides, thioamides, thioureas, and ureas.^{96,99} First, when a carbonyl group is introduced in the α -position with respect to the thio nucleophile (thioacid), the acylating agent generated reacts directly with the amine produced to form an amide (Path b, Figure 13).⁹⁶ The extension of this reaction with the concourse of dithioacids and dithiocarbamic acids, which are prepared in situ from carbon disulfide and organometallic compounds and amines, respectively, led to thioamides and thioureas. Furthermore, the treatment of 2,4-dinitrobenzenesulfonamides with benzene hydroxamic acids in the presence of cesium carbonate led to the corresponding ureas.

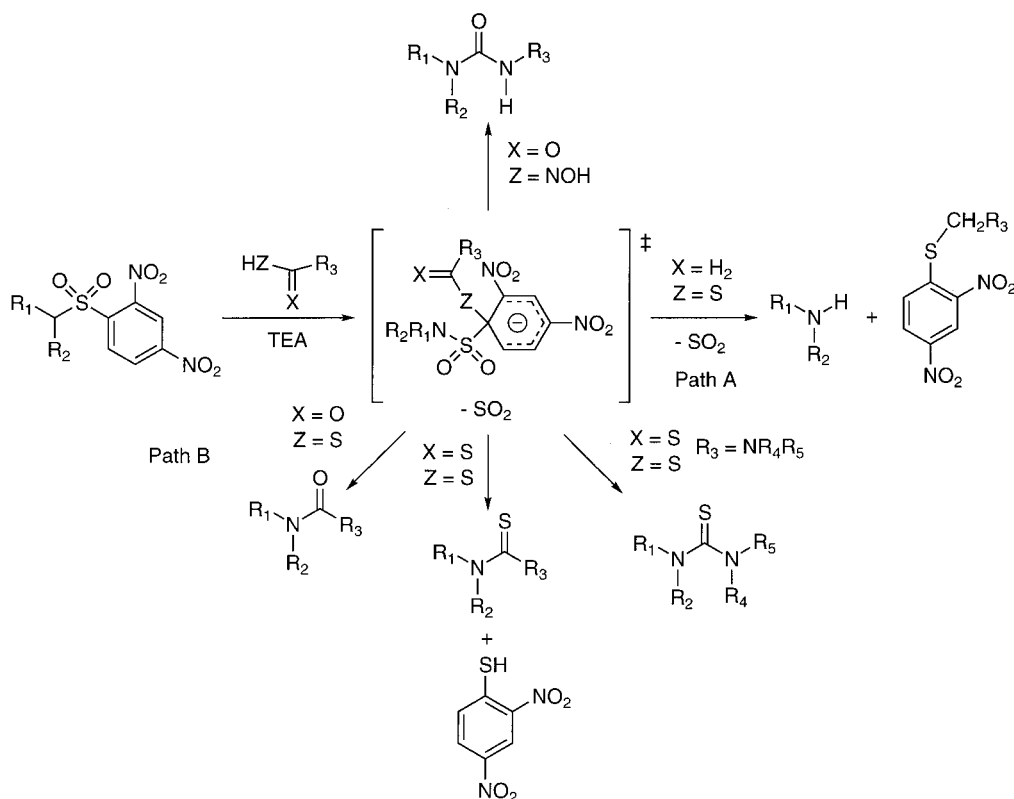


FIGURE 13 Deprotection/functionalization sequence for the one-pot preparation of amides, thioamides, thioureas, and ureas. Adapted from Refs. 96 and 99.

The benzothiazole-2-sulfonyl (Bts) group has been used in solution in a similar manner to the NBS group. *N*-Bts-amino acids are obtained from Bts-Cl using the Schotten–Baumann procedure.^{87,88} Methylation of Bts peptides was achieved using MeI/K₂CO₃ in acetonitrile. Cleavage of Bts from nonmethylated derivatives was achieved using reducing agents such as Al/Hg, Zn, or H₃PO₂.⁸⁷ However, *N*-methylated Bts derivatives proved to be less reactive compared to the Bts-NH analogues, and the deprotection step was more sensitive to concentration effects. For these derivatives, NaBH₄ in ethanol was very effective, but for nonmethylated systems this method failed, apparently due to competing deprotonation of the acidic Bts-NH subunit. Finally, the thiophenol/K₂CO₃ system, which was developed for the NBS derivatives, cleaved both methylated as well as

nonmethylated *N*-Bts derivatives.⁸⁸ DIEA or potassium *tert*-butoxide can be also used as the base.¹⁰⁰ This strategy has been used for the synthesis in solution of a fragment of cyclosporin containing three *N*-methyl-amino acids in a row.⁸⁸

These two protecting groups have been used in combination with *t*-Bu-based protecting groups, but they should also be compatible with Fm derivatives.

THIOL-LABILE PROTECTING GROUPS

The dithiasuccinoyl (Dts) function was conceived to meet the requirements of an orthogonally removable *N*^α-amino protecting group.⁵ Dts-amines resist strong

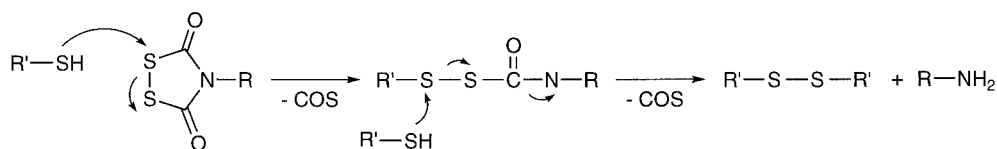


FIGURE 14 Mechanism for the removal of Dts group. Adapted from Ref. 101.

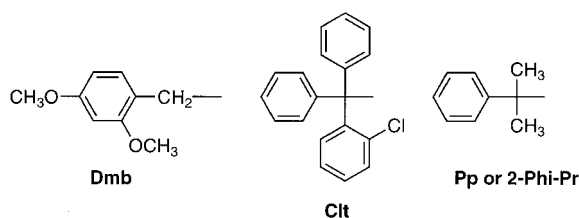


FIGURE 15 Structures of acid-labile protecting groups.

acids and light, but are rapidly and specifically cleaved under mild conditions by thiolysis.¹⁰¹ Thus, the first *three-dimensional* orthogonal protection scheme suitable for the preparation of fully and partially protected peptides involved Dts for N^α -amino protection, *t*-Bu for side-chain protection, and an *o*-nitrobenzyl ester anchoring linkage.¹⁰² The Dts group is cleaved by treatment with thiols such as 2-mercaptoethanol (0.5M)–DIEA (0.5M) in CH₂Cl₂ (2 × 2 min) or *N*-methylmercaptoacetamide (0.5M)–NMM in CH₂Cl₂ (2 × 2 min).^{101,103} Dts-amino acids can be obtained by reaction of either a thiocarbamate or the formyl derivative with chlorocarbonylsulfenyl chloride.^{104,105} They are mostly obtained as oils and therefore need to be isolated and stored as DCHA salts.¹⁰⁴ Although the Dts protecting group has been used in the synthesis of some model peptides,^{102,103} as well as peptide nucleic acid (PNA) oligomers,¹⁰⁶ an as yet unclear side reaction that leads to terminated peptides occurs during the removal of this group and has thus limited its use in SPPS. (See Figure 14.)

The most important feature of Dts is its stability in the presence of acids. The Dts group is stable to TFA, HBr/HOAc, and even 90% HNO₃ at 5°C, a fact that has allowed the regioselective nitration of 4-(dithiasuccinoylaminoethyl)benzoic acid.¹⁰⁵ Another interesting application of Dts has been in glycopeptide solid-phase chemistry,¹⁰⁷ where it has been selectively removed in the presence of an azide by treatment with NaBH₄¹⁰⁸ or 1,3-propanedithiol.¹⁰⁹

C-TERMINAL CARBOXYL PROTECTING GROUPS

As SPPS is typically carried out in the $C \rightarrow N$ direction by sequential incorporation of N^α -urethane protected amino acids followed by removal of the protecting group, the development of temporary protecting groups for the *C*-terminal carboxyl function has not been widely investigated. However, allyl and Fm esters have been used for the temporary protection the *C*-terminal carboxyl function for the solid-phase

assembly in the reverse $N \rightarrow C$ direction^{110,111} as well as for the preparation of “head-to-tail” cyclic peptides.^{65,66,112–114} Furthermore, the allyl group is also compatible with the synthesis of *C*-terminal modified peptides using a BAL strategy.^{24,115–117} Removal of these protecting groups can be achieved in a similar way to that described above for the carbamate derivatives used for N^α -protection. Allyl esters can be easily prepared by refluxing the carboxylic acid with allyl bromide in the presence of DIEA,^{65,112} or with allyl alcohol in the presence of DCC/dimethylaminopyridine (DMAP).⁶⁵ The latter method is also used for the preparation of Fm esters.^{118,119} Alternatively, these esters can be obtained from 9-fluorenylmethylchloroformate in the presence of DIEA/DMAP¹²⁰ or by transesterification of *p*-nitrophenyl esters in the presence of imidazole.¹²¹

Cleavage of 2,4-dimethoxybenzyl (Dmb) esters is achieved with 1% TFA in CH₂Cl₂ and can therefore be performed in the presence of *t*Bu-based side-chain protecting groups as well as TFA-labile resins such as Wang or PAL.¹²² This protecting group has been used for the preparation of “head-to-tail” cyclic peptides in combination with a Fmoc/*t*-Bu strategy.¹²² These esters are obtained from the carboxylic acid and 2,4-dimethoxybenzyl alcohol in the presence of catalytic amounts of acid.¹²²

The 2-chlorotrityl (Clt) group has been used for the semipermanent protection of a *C*-terminal carboxyl group in the synthesis of a fragment of the HIV-1 protease using a convergent approach.¹²³ In this case, the growing peptide was anchored to the solid support through the imidazole ring of a His residue. Removal of the Clt is accomplished with 1% TFA in CH₂Cl₂. These esters are obtained by reaction of the carboxylic acid with Clt-chloride/DIEA in CH₂Cl₂.

The 2-phenylisopropyl (Pp or 2-Phi-Pr)¹²⁴ esters have been used for the ω -protection of Asp and Glu residues in the solid-phase synthesis of cyclic peptides¹²⁵ and as *C*-terminal protecting groups in the synthesis of protected peptide fragments in solution.¹²⁶ As side-chain protecting groups can be removed with 1% TFA in CH₂Cl₂,¹²⁵ while for α -protection treatment with 4% TFA in CH₂Cl₂ for 15 min

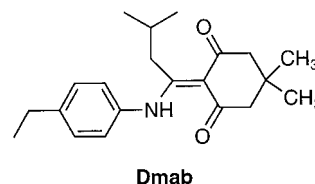


FIGURE 16 Structure of the Dmab group.

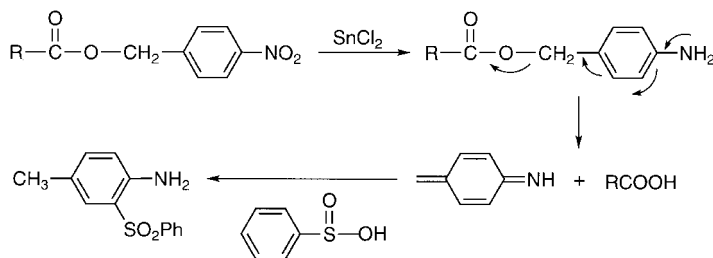


FIGURE 17 Mechanism for the removal of the *p*Nb group. Adapted from Refs. 128 and 134.

is required.¹²⁶ Under these conditions, of Boc or *Or*-Bu was not detected. The preparation of these esters should be carried out from the phenylisopropyltrichloroacetamidate because the use of the alcohol in the presence of DCC/DMAP was unsuccessful.¹²⁶ (See Figure 15.)

The 4-{*N*-[1-(4,4-dimethyl-2,6-dioxocyclohexylidene)-3-methylbutyl]amino}benzyl (Dmab) group,¹²⁷ which is complementary to the *iv*Dde group⁵¹ for amine protection, has been used for both the side-chain protection of Asp and Glu residues, and for the temporary protection of the C-terminal carboxyl function (See Figure 16). Dmab, as well as *iv*Dde, is stable to piperidine and is removed by hydrazine · H₂O–DMF (2:98) for 5 min in a continuous flow system. The deprotection step, which occurs through the formation of the same indazole derivative described for the amino protecting groups, affords a 4-aminobenzyl ester which suffer a spontaneous collapse via 1,6-electron pair shift to render the free carboxyl acid (see Figure 17 for a similar mechanism).

For the preparation of head-to-tail cyclic peptides, the *p*-nitrobenzyl ester (*p*NB) represents an attractive alternative.¹²⁸ These esters are stable to the acid conditions used to remove the Boc group, as well as the small number of cycles of piperidine/DMF used to remove the Fmoc group.^{128,129} The esters can be cleaved by reducing agents, such as Zn in HOAc, Na₂S, Na₂S₂O₄, or SnCl₂ in aqueous organic solvents.^{128,130,131} For the use of *p*NB in the solid phase, SnCl₂ is a very convenient reagent because when dissolved in DMF, a common solvent used in SPPS, the nitro group is easily reduced. However, this reduction leads to the formation of quinonimine methide^{132,133} and this leads to intractable contaminants. Recently, it has been shown that this intermediate can be converted into a sulfone through its reaction with benzene sulfinic acid and the resulting product can be easily removed from the resin.¹³⁴ (See Figure 17.)

The *p*NB group is removed by treatment with 8*M* SnCl₂ in DMF containing 1.6 mM HOAc and 0.2% phenol for 5 h at 25°C.¹³⁴ Alternatively, it can be

removed by three treatments of 30 min at 5–60°C¹²⁸ followed by washings with DMF, MeOH, and DMF, and treatment with 8*M* benzene sulfinic acid in DMF for 30 min at 25°C.¹³⁴ Finally, the resin is washed with DMF and MeOH. The *p*NB esters can be prepared by reaction of the carboxylic acid with *p*-nitrobenzyl bromide in the presence of DIEA using acetone or CH₂Cl₂ as solvent.^{128,134}

The *p*NB group can be used for the protection of the thiol function of Cys and in the form of its carbamate for the protection of amino functions.¹³⁴

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