1
Protection Reactions

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1.1
General Considerations

Peptides, polypeptides, and proteins are the universal constituents of the biosphere. They are responsible for the structural and functional integrity of cells. They form the chemical basis of cellular functions that are based on highly specific molecular recognition and binding, and are involved as key participants in cellular processes. A peptide or a protein is a copolymer of α-amino acids that are covalently linked through a secondary amide bond (called a peptide bond). They differ from one another by the number and sequence of the constituent amino acids. Generally, a molecule comprised of few amino acids is called an oligopeptide and that with many amino acids is a polypeptide (molecular weight below 10 000). Proteins contain a large number of amino acids. Due to the vitality of their role for the function as well as survival of cells, peptides and proteins are continuously synthesized. Biosynthesis of proteins is genetically controlled. A protein molecule is synthesized by stepwise linking of *unprotected* amino acids through the cellular machinery comprised of enzymes and nucleic acids, and functioning based on precise molecular interactions and thermodynamic control. Thousands of proteins/peptides are assembled through the combination of only 20 amino acids (referred to as coded or proteinogenic amino acids). Post-translational modifications (after assembly on ribosomes) such as attachment of nonpeptide fragments, functionalization of amino acid side-chains and the peptide backbone, and cyclization reactions confer further structural diversity on peptides.

The production of peptides via isolation from biological sources or recombinant DNA technology is associated with certain limitations *per se*. A minor variation in the sequence of a therapeutically active peptide isolated from a microbial or animal source relative to that of the human homolog is sufficient to cause hypersensitivity in some recipients. Further, the active drug component is often not a native peptide but a synthetic analog, which may have been reduced in size or may contain additional functional groups and non-native linkages. The development of a drug from a lead peptide involves the synthesis (both by conventional and combinatorial methods) and screening of a large number of analogs. Consequently, the major proportion of the

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demand for peptides is still met by chemical synthesis. Chemical synthesis is also crucial for synthesizing peptides with unnatural amino acids as well as peptide mimics, which by virtue of the presence of non-native linkages are inaccessible through ribosomal synthesis.

Synthetic peptides have to be chemically as well as optically homogenous to be able to exhibit the expected biological activity. This is typically addressed by using reactions that furnish high yields, give no or minimum side-products, and do not cause stereomutation. In addition, the peptide of interest has to be scrupulously purified after synthesis to achieve the expected level of homogeneity. The general approach to synthesize a peptide is stepwise linking of amino acids until the desired sequence is reached. However, the actual synthesis is not as simple as the approach appears to be due to the multifunctional nature of the amino acids. Typically, a proteinogenic amino acid (except Gly) contains a chiral carbon atom to which is attached the amino (α-amino), carboxy, and alkyl group (referred to as the side-chain). Gly lacks the alkyl substitution at the α-carbon atom. Also, the side-chains of many of the amino acids are functionalized.

A straightforward approach to prepare a dipeptide A–B would be to couple the carboxy-activated amino acid A with another amino acid B. However, this reaction will yield not only the expected dipeptide A–B, but also an A–A (through self-acylation) due to the competing amino group of A. The so-formed dipeptides can further react with A since they bear free amino groups and form oligopeptides A–A–B, A–A–A, or A–A–A–A, and the reaction proceeds uncontrollably to generate a mixture of self-condensation products (homopolymers) and oligopeptides of the type A_nB. The process becomes even more complicated when reactive functional groups are present in the side-chains of the reacting amino acid(s). The uncontrolled reactivity of multiple groups leads to the formation of a complex mixture from which it becomes a Sisyphean task to isolate the desired product, which would have been formed, mostly, in low yield. The solution to carry out peptide synthesis in a chemoselective way is to mask the reactivity of the groups on amino acids that will not be the components of the peptide bond prior to peptide coupling step. This is done by converting the intervening functional group into an unreactive (or less reactive) form by attaching to it a new segment, referred to as a protecting group (or protection or protective function). The chemical reactions used for this purpose are known as protection reactions. The protecting groups are solely of synthetic interest and are removed whenever the functional group has to be regenerated. In other words, the protection is *reversible*. In the light of the concept of protection, the steps involved in the synthesis of the above dipeptide A–B are depicted in Figure 1.1.

Protections are employed for α-amino, carboxy, and side-chain functional groups (Figure 1.2). Since peptide synthesis is a multistep and repetitive process, the longevity of different protecting groups on the peptide under synthesis varies. In the present and widely followed approach of assembling peptides, wherein the peptide chain extension is from the carboxy- to amino-terminus (C → N direction), the α-amino protection is removed after each peptide coupling step to obtain a free amino group for subsequent acylation and, hence, this protection is temporary. The carboxy and side-chain protections are generally retained until the entire sequence

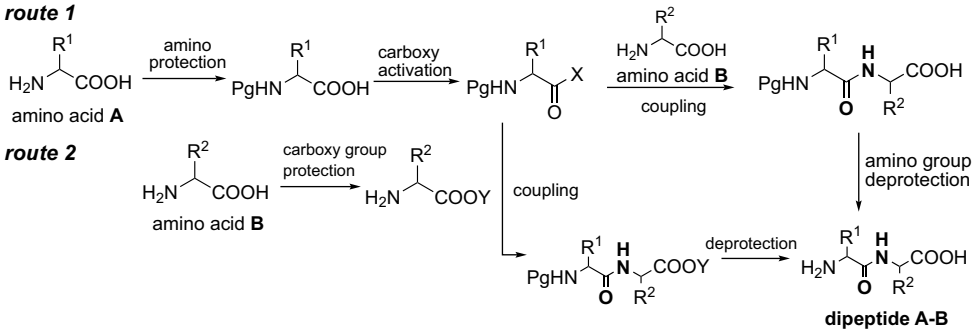


Figure 1.1 Illustration of synthesis of a dipeptide using α-amino and carboxy protections.

is assembled, and are removed simultaneously in a single step at the end of the synthesis. Hence, they can be regarded as semipermanent groups. The transient α-amino protection should be removed using reagents/conditions that do not affect the stability of semipermanent groups and, importantly, the newly assembled peptide bond(s). Consequently, it should be orthogonal to semipermanent groups with respect to its susceptibility to a particular cleavage reaction. Sometimes it may be required to remove only the carboxy protection or a particular side-chain protection in order to obtain a N^{α}-protected peptide acid or to regenerate a side-chain functional group (for site-selective peptide modification). In such cases, the α-amino and semipermanent groups have to be orthogonal to one another.

In practice, the orthogonality among protecting groups is achieved by either differential reactivities or different rates of reaction of protective units towards a particular cleavage reagent. The compulsion for the requirement of semipermanent groups can be lifted especially with respect to the protection of side-chain functionalities if there is no possibility of an undesired reaction from the unprotected group during coupling or deprotection of the α-amino group. Hence, the degree of protection can widely vary (from maximum to minimum) depending upon the synthetic design and the choice of chemistry.

An ideal protecting group should be quantitatively introduced and removed (desirably using mild reagents/conditions), should leave no residue nor form a byproduct that is difficult to separate from the product, should not be prematurely deblocked or modified during synthesis, and should not cause side-reactions including stereomutation. In addition, it should not influence the reactivity of the adjacent groups or, if it does, it should be in predictable ways.

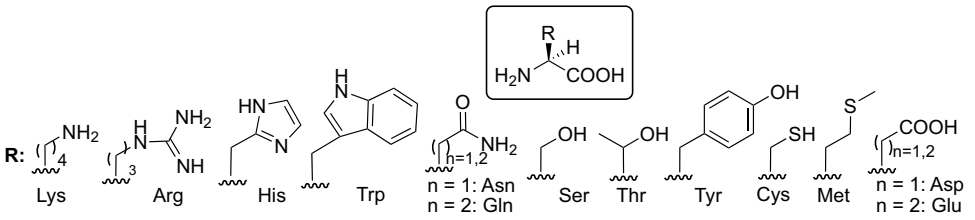


Figure 1.2 Side-chain functional groups of amino acids that entail protection.

In this chapter, various α-amino, carboxy, and side-chain protecting groups are presented. The general features of each type of protecting groups, methods of introduction and removal, and improved analogs are discussed. Typical and widely used preparative methods are mentioned under each category of protecting groups. The reader may refer to many earlier works for accounts on the development of protecting groups and for detailed discussions on different aspects of protecting group chemistry in peptide synthesis [1].

1.2 α-Amino Protection (N^{α} Protection)

The α-amino group is protected to reduce its nucleophilicity. In addition to the general properties of a protecting group, an ideal α-amino protection is expected to possess more properties unique to itself. Deblocking of the N^{α} protection should take place with a high degree of selectivity so that there will be no progressive loss of the semipermanent groups with repetitive deblocking steps as the peptide chain is elongated. The N^{α} protection should not sterically or electronically disfavor the reactions at the carboxy group by virtue of its proximity. It should not be involved or promote side-reactions, including those that lead to stereomutation. Further, it should form stable and crystallizable amino acid derivatives. Indeed, due to such stringent requirements for a α-amino protecting group, the success in the development of a good N^{α} protection has always been critical to progress in the development of efficient coupling methods and, in turn, to the overall growth of the field of peptide synthesis.

The α-amino protections are of different types and they can be categorized using different approaches. However, based on the criteria of the magnitude of the present utility of each type, the groups can be classified into non-urethane- and urethane-type *N* protections. Presently, the latter are the extensively used N^{α}-protecting groups for both solution and solid-phase peptide synthesis (SPPS) due to reasons that will be discussed later. The extent of the utility of the non-urethane-type amino protectors in peptide synthesis is currently comparatively lesser. Only a few groups of this category have been demonstrated to be efficient as N^{α}-protectors for general applications. Nonetheless, they are useful as protecting groups for side-chain functions as well as for the protection of the α-amino group for the synthesis of peptide mimics and unnatural amino acids. Their importance in peptidomimetic synthesis owes much to the vast diversity in chemistry required for accomplishing a wide range of backbone modifications of peptides leading to novel nonpeptidic molecules.

1.2.1 Non-Urethanes

1.2.1.1 Acyl Type

Reaction of amino acids with alkyl or aryl carboxylic acid derivatives yields *N*-acyl amines or amides. Acyl groups were the first generation of N^{α}-protecting groups used for peptide synthesis. The necessity for the protection of the α-amino group for

successful peptide synthesis was identified as early as 1900s by the two distinguished chemists of the time, Emil Fischer and Theodor Curtius, who mostly employed formyl (For), acetyl (Ac), and benzoyl (Bz) groups for this purpose. However, it was soon realized that the selective removal of these protections from peptides was not successful. The acyl groups present two synthetic difficulties in general – difficulty in the removal of the group without destroying the meticulously assembled peptide bonds and a high degree of racemization of N^{α}-acyl-protected amino acid derivatives. The only mode of deprotection of an acyl-protected amine is the fission of the acyl-nitrogen (-CO−NH-) bond. However, since the peptide bonds (secondary amides) are chemically similar to the amide bond (of the protective function), they are often simultaneously cleaved. Although selective removal of the N^{α}-acyl group has been attempted through special methods such as the enzymatic and CNBr-mediated cleavage of N-terminal Z-Arg and Met peptides, respectively, these protocols have not found widespread application. However, if the N^{α}-acyl group contains an electron-withdrawing substitution (e.g., CF_3CO-, trifluoroacetyl (Tfa) group), then the amide carbonyl of the protective function becomes more susceptible to nucleophilic substitution relative to the peptide carbonyl and thus the amino group can be selectively deprotected under acceptable conditions. Selectivity can also be achieved by using groups that can be modified (postcoupling) into units, which can be eliminated through processes such as lactam formation. Barring these examples, simple acyl groups do not find established applications as α-amino protections for conventional peptide synthesis. Nonetheless, the For protection can be attributed with a unique application. The N^{α}-formyl group of protected amino acid esters/amides and peptide esters **1** can be readily dehydrated into the isocyano group and the resulting α-isocyano esters/amides **2** can be used as key components to synthesize peptides and peptide libraries through multicomponent reactions (MCRs). MCRs have been shown to be particularly useful to assemble peptides linked by sterically hindered amino acids such as α,α-dialkylamino acids. For instance, an extremely difficult sequence **4** with three successive α,α-diphenylglycine (Dph) units has been assembled through a modified Ugi reaction of isonitrile **3** with Z-Dph-OH and diphenylmethanimine (Figure 1.3) [2]. Mild and racemization free conversion of N^{α}-For-protected amino acid and peptide derivatives into isonitriles can be carried out by the treatment with triphosgene in dichloromethane (DCM) at −75 to −30 °C (Figure 1.3) or Burgess reagent [3].

1.2.1.1.1 Monoacyl Groups

Trifluoroacetyl (Tfa) Group Tfa is of special interest as a monoacyl-type protecting group. Due to the negative inductive effect of the $-CF_3$ substitution, the trifluoroacetamides readily undergo hydrolysis in mild alkaline conditions to which peptide bonds and most carboxy esters are largely stable, not withstanding methyl and ethyl esters (which are susceptible to saponification). Optically pure N^{α}-Tfa-amino acids are prepared by treating amino acids with trifluoroacetic anhydride (TFAA) in anhydrous trifluoroacetic acid (TFA) solvent at −10 to + 10 °C [4]. The method can also be successfully used to obtain N^{α}-Tfa-Lys/Orn from Lys/Orn. The acidity of the

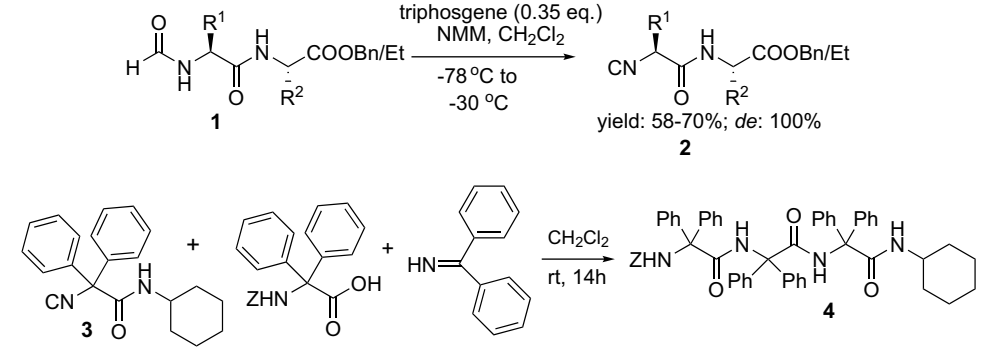


Figure 1.3 Synthesis of isocyanato peptides from *N*-For-protected peptide esters and the Ugi four-component reaction of α-isocyanato esters.

medium protonates the more basic ω-amino group of Lys/Orn into the ammonium form, which do not undergo acylation. However, the strong acidic condition is disadvantageous in the case of preparation of Tfa-Ser-OH and Tfa-Thr-OH as these hydroxy amino acids are dehydrated into unsaturated amino acids. Trifluoroacetylation can also be carried out using ethyl thioesters and phenyl/alkyl esters of TFA such as ethyl trifluoroacetate [4] or reagents such as 1-(trifluoroacetyl)imidazole. The N^{α}-Tfa group is cleaved by the action of 0.2 N NaOH [5] or $Ba(OH)_2$ or by dilute NH_3 solution. Piperidine [6] and $NaBH_4$ in EtOH can also be employed. The group is resistant to acids except for Tfa-Ser/Thr derivatives in which it is cleaved by mild acidic reagents. However, strong acidic conditions such as boiling methanolic HCl can cleave the group.

1.2.1.1.2 **Groups Cleavable via Lactam Formation** 2-(4,5-Dimethyl-2-nitrophenoxy)-2-methylpropionyl group **5a** and its phenyldiazenyl analog **5b** are introduced by the reaction of the corresponding acid chlorides with amino acids. Cleavage is accomplished in two steps (Figure 1.4). The first step is the reduction of the nitro group into an amino group by catalytic hydrogenation or catalytic transfer hydrogenation (CTH). Step 2 is the cyclization of the resulting amino compound **6** into a lactam **7** at neutral pH with concomitant elimination of the protected amine [7]. A similar process also cleaves **5b** [8]. Nevertheless, incomplete reduction and cyclization steps have been the major concerns for a broad application of these groups in spite of selective and acceptable cleavage conditions.

Racemization The high degree of racemization of N^{α}-acyl-protected amino acids has been attributed to the facile formation of optically labile azlactone intermediates

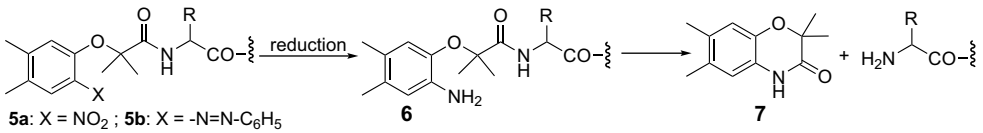


Figure 1.4 Cleavage of N^{α} protection via lactam formation.

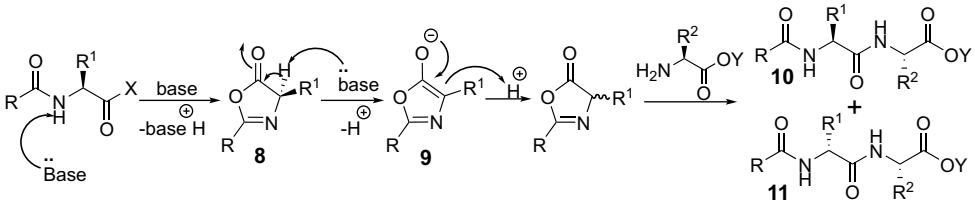


Figure 1.5 Racemization of N^{α}-acyl-α-amino acid derivatives.

8. Activated N^{α}-acyl amino acids readily undergo base-catalyzed ring closure to azlactones (2,4-disubstituted oxazol-5(4*H*)-ones). Enolization of the latter to oxazol-5-ol **9** in the presence of a base results in the loss of chirality at the α-carbon atom. Azlactones can acylate amines, but the resulting product will be a mixture of epimeric peptides (**10** and **11**, Figure 1.5). In the case of *N*-methyl-α-amino acids (NMAs), the oxazolium intermediate can be formed even in the absence of base, due to the electron-releasing effect of the *N*-alkyl substitution. Hence, N^{α}-acyl, N^{α}-alkylated amino acid derivatives are extremely sensitive to racemization during coupling. Base-catalyzed enolization of the activated amino acid derivatives with the abstraction of the α-proton also contributes to racemization.

Racemization can also take place during the introduction of N^{α}-acyl protection because of the *in situ* activation of the carboxy group by acid anhydrides and acid chlorides (used as reagents for acylation of α-amino group) followed by cyclization to azlactones. For instance, the N^{α}-Tfa-amino acids prepared by the treatment of amino acids with an excess of TFAA in the absence of TFA have been found to be contaminated with the D-isomer. This is due to the activation of Tfa-amino acids by TFAA to unsymmetrical or symmetrical anhydrides, which rearrange with racemization to the corresponding Tfa-azlactones.

1.2.1.1.3 **Diacyl Groups** Reaction of amino acids with 1,2-dicarboxylic acid derivatives yields imides that are stable to acids and also to hydrogenolysis, thus making the diacyl-type protection suitable for usage in diverse synthetic conditions. These groups are cleaved by nucleophilic substitution by hydrazine or thiols. The aromatic 1,2-dicarboxylic acid, phthalic acid, is employed for N^{α} protection, whereas the alkyl counterpart *N*-maleoyl group has been replaced by the dithiasuccinoyl (Dts) group.

Phthaloyl (Phth) Group N^{α}-Protected Phth-amino acids **12** are prepared under mild and racemization-free conditions by using phthaloylating reagents (Figures 1.6 and 1.7) such as *N*-(ethoxycarbonyl)phthalimide **13**, monoethyl phthalate **14** [9], and

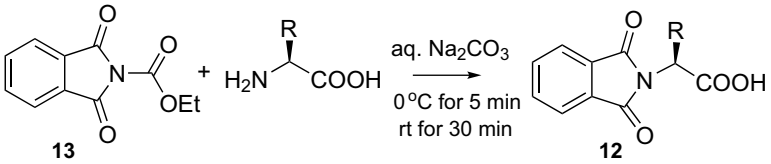


Figure 1.6 Preparation of N^{α}-Phth-amino acids.

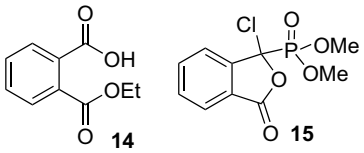


Figure 1.7 Phthaloylating reagents.

3-chloro-3-(dimethoxyphosphoryl)isobenzofuran-1(3*H*)-one **15** [10]. *N*-Phthaloylation by these reagents has almost completely replaced the original and harsh route of fusing amino acids with phthalic anhydride, which invariably caused racemization. An improvement in the method was achieved by using solvents such as benzene, dioxane, and so on, but could not overcome the racemization problem completely.

The N^{α}-Phth group is normally removed by means of hydrazinolysis by treatment with hydrazine hydrate in refluxing MeOH or EtOH [11]. Alternatively, a two-step procedure, which involves a reductive ring opening, followed by an acid-catalyzed lactonization of the resulting hydroxy compound (**17**) with concomitant fission of acyl-nitrogen bond, has also been developed (Figure 1.8) [12]. Interestingly, Phth protection cannot be removed by treatment with alkali. The alkali opens the five membered ring to a monoacyl amide of phthalic acid **18** (*O*-carboxybenzoyl amide) which is stable to hydrazine and to bases, thus representing an irreversible protection. Hence, saponification cannot be used as a method to cleave esters of N^{α}-Phth-protected peptide acids. On the other hand, treatment with $SOCl_2$ or methanolic HCl converts **18** back to phthalimide. In fact, this cyclization has been used as the basis for the development of a mild protocol for preparation of phthalimides. Tetrachlorophthaloyl group is an improved analog of Phth and can be removed under mild conditions by treatment with 15% hydrazine in *N*,*N*′-dimethylformamide (DMF) for 1 h at room temperature [13].

Groups Removed by Reductive Cleavage Dithiasuccinoyl (Dts) imides are stable to acids and to photolysis, and are cleaved by reductive thiolysis. N^{α}-Dts-amino acids **19** are prepared through a multistep route, which involves the reaction of the *tert*-butyl esters of amino acids with alkyldithiocarbonate or trithiodicarbonate to form

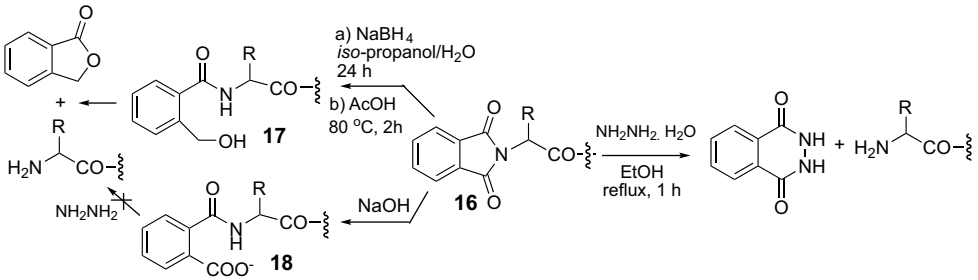


Figure 1.8 Cleavage of the N^{α}-Phth group.

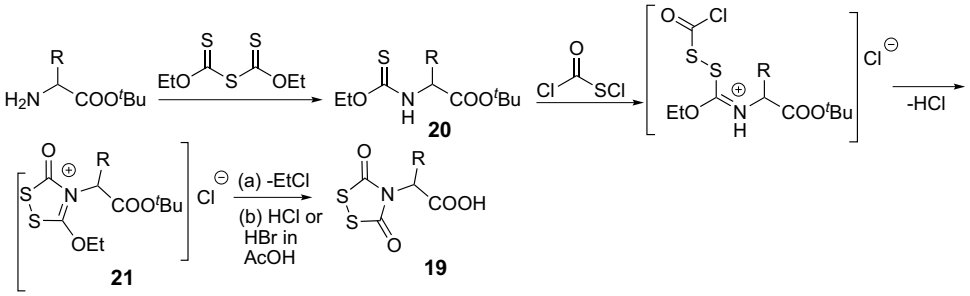


Figure 1.9 Preparation of N^{α}-Dts-protected amino acids.

N^{α}-ethoxythiocarbonyl amino acid esters **20**. The latter, upon treatment with chlorocarbonylsulfanyl chloride (Cl−CO−SCl), forms a cyclic intermediate **21**, which eliminates chloroethane to yield *N*-Dts-imides (Figure 1.9). The final step is the acidolytic cleavage of the *tert*-butyl ester [14]. Alternatively, a one-pot procedure based on the treatment of amino acids with polymeric poly(ethylene glycol) (PEG)-xanthane (PEG-OCSSCH$_2$CONH$_2$) has been developed [15], which also circumvents the generation of carbamate impurities by reaction with Cl−CO−SCl in the former method. Dts protection is cleaved within minutes by mercaptoethanol in DCM in the presence of triethylamine (TEA) or diisopropylethylamine (DIPEA) [16] (Figure 1.10).

The group can also be removed by using *N*-(methylsulfanyl)acetamide, trialkylphosphines and hydride donors. It is noteworthy that the Dts group is deblocked through a process initiated by nucleophilic attack on the sulfur atom adjacent to the amide carbonyl unlike the other acyl-type protections wherein the cleavage is due to nucleophilic attack at the amide carbonyl. Hence, in the case of this acyl-type protection, a cleavage reagent can selectively act at the protection unit and not at peptide bonds.

The N^{α}-(alkyldisulfanyl)carbonyl groups **22** and **23** are cleaved similarly by thiols and trialkylphosphines (Figure 1.11) [17]. Hence, these groups represent useful monoacyl-type protections.

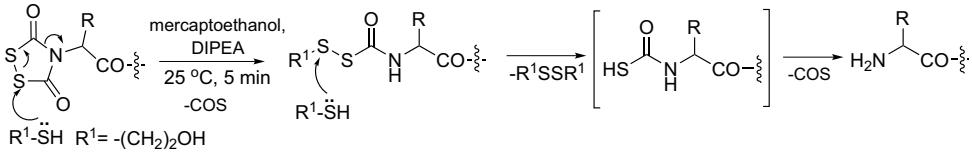


Figure 1.10 Thiolytic cleavage of N^{α}-Dts protection.

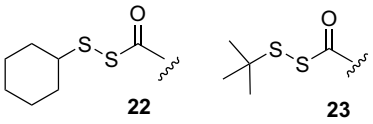


Figure 1.11 Monoacyl protections cleaved by thiolysis.

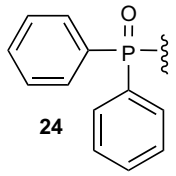


Figure 1.12 Dpp group.

1.2.1.2 **Phosphine-Type Groups**

Phosphine groups such as the diphenylphosphine (Dpp) group **24** (Figure 1.12) are stable to bases and catalytic hydrogenation, and sensitive to acids. They differ from the other acid-labile protections (e.g., triphenylmethyl (trityl or Trt), *tert*-butoxycarbonyl (Boc)) in that the acidolytic cleavage of the group does not result in the formation of carbocations which can cause undesired alkylations (see below). The Dpp group has been successfully employed in peptide synthesis. N^{α}-Dpp-protected amino acids are prepared by treating amino acid methyl esters with diphenylphosphinic acid chloride (Dpp-Cl) followed by alkaline hydrolysis of the ester. The protection is removed by treatment with 2 equiv. of 4-toluenesulfonic acid (TsOH) in MeOH (1–6 h) or 6 equiv. of HCl in MeOH (2–3 h) [18].

1.2.1.3 **Sulfonyl-Type Groups**

Reaction of amino acids with aryl/alkylsulfonic acid derivatives yields the corresponding sulfonamides. The 4-toluenesulfonyl (tosyl or Ts) group **25** is the first example of this type, which was described by Emil Fischer. However, its application to peptide synthesis has been constrained due to the difficulties such as cumbersome removal conditions (the only method of cleavage is reduction with sodium in liquid NH_3), high reactivity of the sulfonamide nitrogen (source of a number of side-reactions such as N^{α}-alkylation), and rapid hydrolysis of Ts-Gly peptides. Hence, the Ts group has been replaced by the more efficient 2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-sulfonyl (Pbf) **26** [19], 2-nitrobenzenesulfonyl (Nbs) **27** [20], 4-nitrobenzenesulfonyl (nosyl) **28** [21], 2-(trimethylsilyl)ethanesulfonyl (SES) **29** [22], and *tert*-butylsulfonyl (Bus) **30** groups (Figure 1.13) [23].

N^{α}-Protected Pbf amino acids are prepared by the action of Pbf-Cl on amino acids under Schotten–Baumann conditions. The group is stable towards bases and catalytic hydrogenation, and cleaved by 10% dimethyl sulfide (DMS) in TFA. Nbs and nosyl groups are typically deblocked by 5% thiophenol in DMF and mercaptoacetic acid/

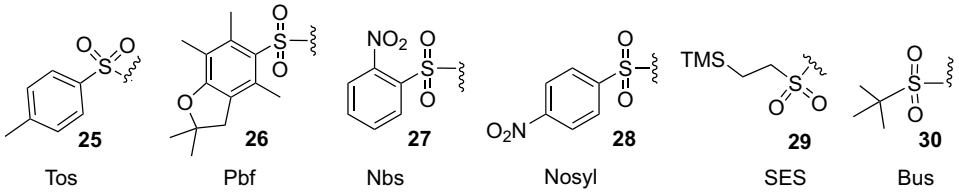


Figure 1.13 Sulfonyl-type α-amino protections.

sodium methoxide in CH_3CN, respectively. The nosyl group on *N*-methylated α-amino groups is deblocked much more readily than that on unsubstituted α-amino groups. SES groups derived from alkylsulfonic acids are stable even towards strong acidic conditions (boiling TFA, 6 M HCl in refluxing tetrahydrofuran (THF), $BF_3{\cdot}O(C_2H_5)_2$) as well as to alkali. The group is cleaved by treatment with cesium fluoride. Notably, the C–Si bond of the group is stable to desilylating reagents, which cleave other silyl protections, particularly the *O*-silyl groups. Hence, SES protection can be used in combination with silyl ethers. The N^{α}-Bus group is introduced by treatment of amino acids with *tert*-butylsulfanyl chloride followed by oxidation using *m*-chloroperbenzoic acid (mCPBA). It is removed using 0.2 N TfOH in DCM in the presence of anisole at 0 °C. N^{α}-Sulfonyl-protected amino acid derivatives are not able to rearrange to oxazol-5-ones even when the α-carboxy moiety is highly activated (e.g., as acid chlorides), thus precluding the possibility of racemization. Also, the N^{α}-sulfonyl-protected amino acid halides are more reactive compared to their carbamoyl counterparts due to the increased inductive effect of the sulfonyl unit. Hence, extremely difficult coupling of sterically hindered amino acids (e.g., MeAib to MeAib for aminoisobutyric acid) has been satisfactorily accomplished with good yields using Pbf-MeAib-Cl [19].

1.2.1.4 **Alkyl-Type Groups**

Alkylation increases the nucleophilicity of amines, in contrast to the primary requirement of a protection to diminish it. Consequently, monoalkylated amines with simple aliphatic *N*-substitutions are seldom protected. Nonetheless, bulky *N*-alkyl groups suppress the reactivity of the amine through steric hindrance. Hence, the α-amino group can be protected by placing crowded groups like Trt and benzhydryl on it. This type of protection is advantageous since the activated N^{α}-alkyl-amino acids do not racemize under standard peptide coupling conditions, as the bulkiness of the protection prevents the abstraction of the α-proton by a base. However, an innate limitation of the method is that the bulkiness of the N^{α} protection can sterically disfavor reactions at the carboxy end, thereby making incorporation of N^{α}-alkyl-amino acids into peptides a difficult task.

1.2.1.4.1 **Triphenylmethyl (Trityl or Trt) Group** N^{α}-Trt-amino acids **31** can be prepared by treating amino acid methyl esters with Trt-Cl followed by alkaline hydrolysis of the ester. Hydrolysis is rather sluggish due to steric hindrance by the Trt group. Alternatively, the amino acids can be directly treated with Trt-Cl (or a more efficient Trt-Br) followed by methanolysis of the *N*,*O*-bis-Trt intermediate (Trt ester) [24]. Formation of Trt esters can be circumvented by using *N*,*O*-bis-trimethylsilyl (TMS) amino acids (Me_3Si-NH-CHR-COO$SiMe_3$), and trisilyl derivatives of Ser, Thr, and Tyr as substrates for tritylation (Figure 1.14) [25]. N^{α}-Trt-amino acids are isolated as stable diethylammonium salts. The Trt group is stable to bases. It is cleaved by mild acids such as 1% TFA or 3% trichloroacetic acid (TCA) in DCM, 0.1 M 1-hydroxy-1*H*-benzotriazole (HOBt) in trifluoroethanol (TFE), or moist 0.2% TFA in DCM [26–28]. The latter two conditions are compatible with acid-labile linkers in SPPS. The group can be preferentially cleaved in the presence of other acid sensitive groups like

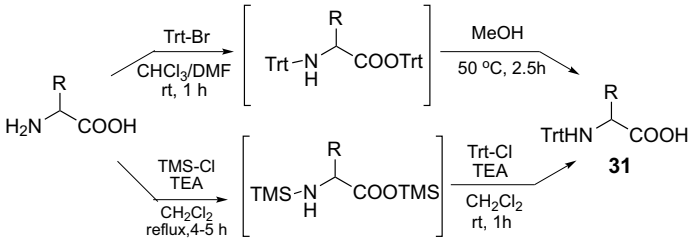


Figure 1.14 Preparation of N^{α}-Trt-protected amino acids.

2-(biphenyl-4-yl)prop-2-yloxycarbonyl (Bpoc) and Boc by pH-controlled titration with HCl in aqueous TFE [29]. Realkylation of the amine during cleavage is prevented due to protonation of the amine in the acidic medium and most effectively by the use of reducing silanes like triethylsilane or even MeOH and TFE. Catalytic hydrogenation and reduction with Na/liquid NH_3 also remove the group.

1.2.1.4.2 **Benzhydryl Groups** The N^{α}-benzhydryl groups (e.g., dibenzosuberyl (Sub) group (Figure 1.15) **32**) are more stable to acids than the Trt group and in addition offer lesser steric hindrance to peptide coupling [30].

1.2.1.4.3 ***N,N*-Bis-Benzyl Protection** *N,N*-bis-Benzyl amino acids are typically prepared by treating amino acids with benzyl chloride in the presence of K_2CO_3. The major product is the *N,N*-bis-benzyl amino acid benzyl ester, which is subjected to alkaline hydrolysis to obtain the free acid. *N,N*-bis-Benzyl protection is preferred to *N*-urethane protections in diastereoselective addition reactions of N^{α}-protected amino aldehydes due to the high rate of racemization in *N*-urethane protected versions [31].

1.2.1.4.4 **Vinyl Groups** 1,3-Diketones (R-CO-CH_2-CO-CH_3) such as acetylacetone, benzoylacetone, and acetoacetic acid ester or the cyclic diketone, 5,5-dimethylhexa-1,3-dione (dimedone) condense with amino acids to give the corresponding *N*-enamine derivatives (R-CO-CH=C(CH_3)-NH-CHR^1-CO-Y). The 1-methyl-3-oxo-3-phenylprop-1-enyl (Mbv) group (R = Ph), can be introduced by condensing amino acids with benzoylacetone in methanolic KOH. The products are isolated as potassium or dicyclohexylamine (DCHA) salts [32]. Acidification of the salts to generate free carboxylic acids is difficult due to high acid sensitivity of the group. However, the Tfa analogs (R = CF_3) can be isolated as free acids by acidification [33]. The N^{α}-Mbv group can be removed by treatment with dilute AcOH or 0.4 M HCl in THF or 0.1 M

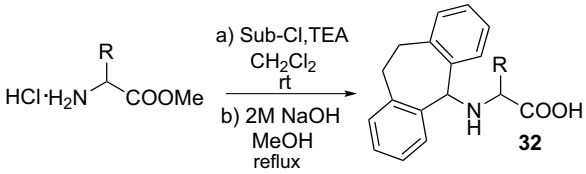


Figure 1.15 Preparation of N^{α}-Sub-amino acids.

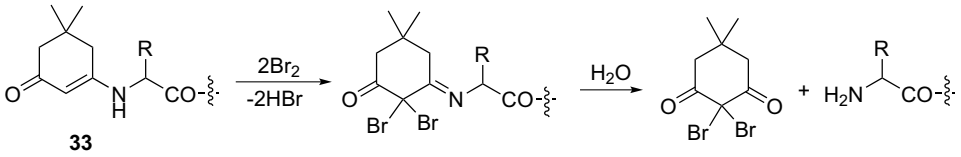


Figure 1.16 Cleavage of the N^{α}-Dim group by bromination.

TosOH in THF [34]. The N^{α}-vinyl derivatives are not prone to racemization. An additional advantage of vinyl-type protections is that the acid-catalyzed hydrolytic cleavage of the protection regenerates the 1,3-dioxo compound that can be recovered and reused. In contrast to the above acid-labile *N*-vinyl groups, the 5,5-dimethyl-3-oxocyclohexen-1-yl (Dim) group **33** is stable to acids and also to hydrogenolysis. It is removed by treatment with bromine water (Figure 1.16) or nitrous acid in AcOH [35]. These conditions can cause bromination and nitrosation of Tyr residues. 1-(4,4-Dimethyl-2,6-dioxocyclohexylidene)ethyl (Dde) and 1-(4,4-dimethyl-2,6-dioxocyclohexylidene)-3-methylbutyl (ivDde) groups are the other vinyl-type protections which are more useful for the protection of ω-NH_2 of Lys.

1.2.1.5 **Sulfanyl-Type Groups**

The N^{α}-sulfanyl groups such as the 2-nitrophenylsulfanyl (Nps) group provide protection against racemization and also do not offer a disfavorable steric effect for peptide coupling. N^{α}-Nps-amino acids **34** are prepared by the reaction of Nps-Cl **35** with amino acids in the presence of a base [36]. Alternatively, Nps-Cl can be converted into a more stable Nps-SCN **36** (by treatment with NaSCN) and then it is used for introducing the group (Figure 1.17) [37]. Crystalline solids of Nps-amino acids are obtained as DCHA salts. The extreme acid stability of the group necessitates special precautions for handling of the free carboxylic acids. Nps groups can be selectively cleaved in the presence of acid-labile *tert*-butyl-based groups by using HCl or HBr in alcohol [38] or in aprotic solvents such as EtOAc or DMF [39]. The acidolytic fission of the sulfenamide bond gives rise to the free amine as well as Nps-Cl, which can cause reattachment of the group. In alcoholic solvents, this is prevented by the conversion of Nps-Cl intermediate into a sulfenic acid ester liberating 1.0 equiv. of HCl that protonates the deblocked amine. However, a similar kind of deactivation of Nps-Cl is

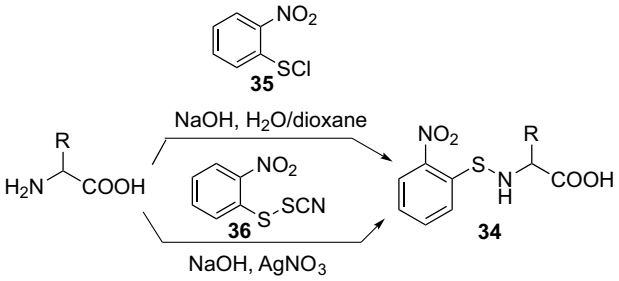


Figure 1.17 Preparation of N^{α}-Nps-protected amino acids.

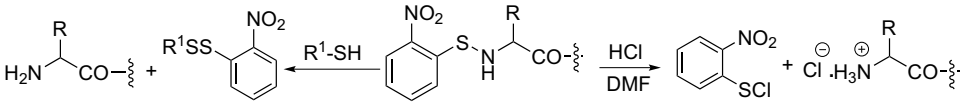


Figure 1.18 Cleavage of the N^{α}-Nps group.

not possible in aprotic solvent. In this case an additional equivalent of HCl is added to protect the deblocked amine as its hydrochloride salt. Scavengers such as 2-methyl indole and 1-acetyl tryptophan are added to decrease the activity of the hydrogen halide in alcohol, and thus protect other acid-labile groups. Alternatively, the group can be cleaved without the risk of formation of Nps-Cl intermediate through thiolysis (Figure 1.18).

1.2.2
Urethanes (Carbamates or Alkyloxycarbonyl Groups)

In 1932, Bergmann and Zervas introduced the benzyloxycarbonyl (Cbz or Z) group as a new amino protecting group [40]. This event not only led to a new epoch in the history of peptide synthesis, but also introduced a new perspective to the conception of protecting group chemistry in organic synthesis as a whole. It spark-started the era of modern peptide synthesis. Until then the practice of peptide synthesis largely relied on the use of acyl groups for α-amino protection, whose selective removal without hydrolyzing the painstakingly assembled peptides was not always possible. In order to circumvent the problem of instability of peptide bonds in harsh deblocking conditions of N^{α}-acyl groups, an approach of peptide chain extension from the amino-terminus (N → C direction) was inevitably followed. Although, repetitive deblocking of N^{α} protection could be avoided, the strategy offered several synthetic difficulties *per se*. Consequently, it was not possible to extend the peptide chain beyond a few amino acid units. The new group (Z group) of Bergmann and Zervas was a urethane-type protection that could be removed, similar to benzyl esters, by catalytic hydrogenation, against which the peptide bonds and alkyl esters were completely stable. It was stable to most of the coupling methods. Later, it was found that the group could be selectively and quantitatively removed by acidolysis too. Addition of these new dimensions of N^{α}-deprotection provided the much-needed stimulus to step up the practice of peptide synthesis to the extent of successfully synthesizing polypeptides. It was also established that the N^{α}-urethane-protected amino acids were less prone to racemization than were the acyl-protected counterparts. The impact of the introduction of this new type of protection on peptide synthesis was so enormous that in only a few years a large number of biologically active peptides as well as several hundreds of their analogs were synthesized. It also initiated studies on the discovery of new urethane protections principally orthogonal to the Z group. Currently, a plethora of urethane protections and a large number of deprotection methods are available.

Urethanes **37** can be regarded as esters of carbamic acids (although the latter are not stable), and the urethane linkage as a hybrid of ester and amide bonds. Due to this

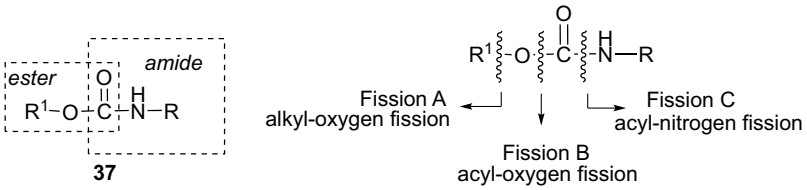


Figure 1.19 Urethane protection: structure and modes of fission.

structure, unlike simple *N*-acyl amines, there exists more than one bond whose fission can result in the deprotection of the amine (Figure 1.19).

Fissions B and C are less likely due to the low reactivity of urethane carbonyl to nucleophiles. Fission A (alkyl-oxygen fission) is the most probable pathway leading to deprotection of the urethane protected amine. It generates the carbamic acid

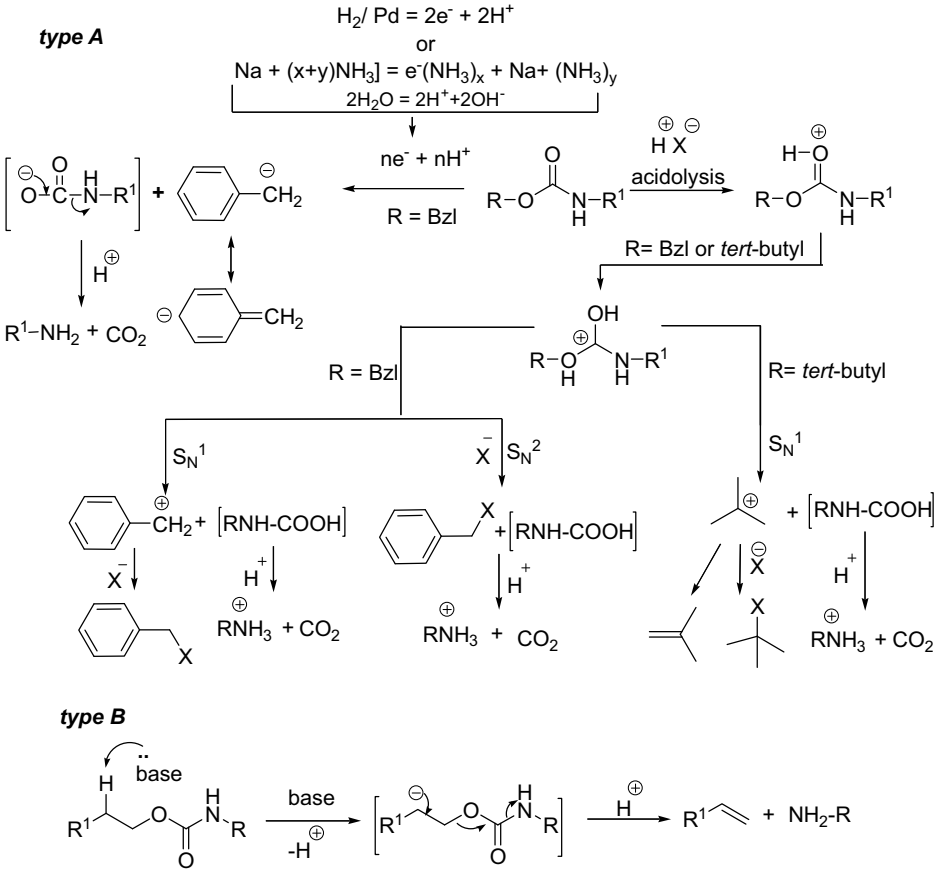


Figure 1.20 Reaction conditions for cleavage of urethane protections. Type A: benzyl and *tert*-butyl urethanes. Type B: urethanes cleaved via β-elimination. (Compiled from [1d].)

that spontaneously decomposes to the amine liberating CO_2. This fragmentation is facilitated by the formation of inductively or resonance-stabilized carbocations or carbanions of the ruptured alkyl fragments (e.g., benzyl- and *tert*-butyl-based urethanes). The important reactions that bring about alkyl-oxygen fission are shown in Figure 1.20 (type B). The reaction mechanism and the type of intermediates formed are also depicted. Alkyl-oxygen fission is also possible via a β-elimination pathway (E_1C_B mechanism) when an acidic methylene group is present β to the oxycarbonyl unit (Figure 1.20, type B). Abstraction of a proton by a base generates a resonance-stabilized carbanion that undergoes an electron shift to form a double bond with the elimination of the oxycarbonyl group, which further loses CO_2 to release the amine (e.g., 9-fluorenylmethyl- and 2-sulfonylethyl-based urethanes).

1.2.2.1 Formation of the Urethane Bond

Urethanes can be considered as the product of a reaction between the components shown in Figure 1.21. The order of incorporation of the components can be different. It can be through an initial formation of a chloroformate followed by its aminolysis (route 1) or through the formation of an isocyanate followed by its alcoholysis (route 2).

In either of the modes, the variable component is only the alcohol. Therefore, a wide variety of urethanes can be prepared by changing the alcohol component. Indeed, the properties of N^{α}-urethane-protected amino acids, such as stability, solubility, methods of cleavage, and reactivity, depend on the nature of the alcohol component of the urethane segment. Hence, in this treatise, the important urethane-type protections are presented according to the structure of the alcohol component.

1.2.2.2 Urethanes Derived from Primary Alcohols

1.2.2.2.1 Benzyloxycarbonyl (Cbz or Z) Group Since its introduction, the Z group has been the most widely employed N^{α} protection for peptide synthesis preferably for solution-phase synthesis. The stability of N^{α}-Z-amino acids, facile introduction and removal conditions (with formation of easily removable cleavage products), and minimum side-reactions of the Z-protected amino acid derivatives have contributed to the widespread utility of this group. The Z group has retained its popularity even to date and it continues to be the protection of choice for peptide synthesis.

Preparation Z-Amino acids **38** can be prepared by acylation of amino acids with benzyl chloroformate **40** (or Z-Cl). The reaction is carried out in the presence of

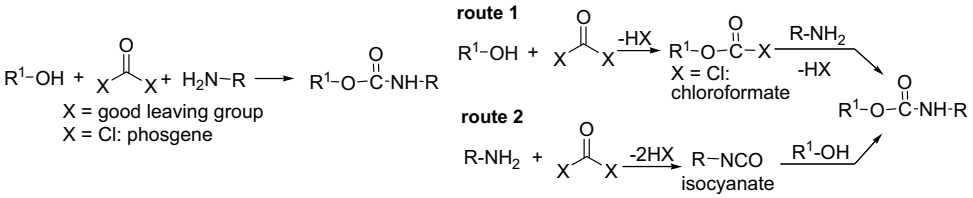


Figure 1.21 Routes to urethane bond formation.

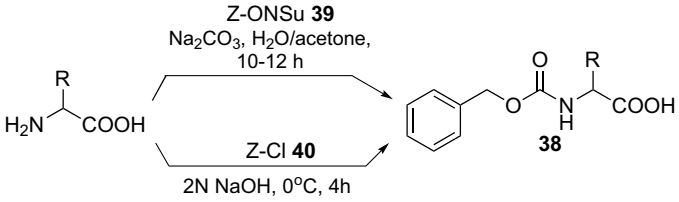


Figure 1.22 Preparation of N^{α}-Z-amino acids.

Na_2CO_3 or NaOH in an aqueous–organic mixture (Schotten–Baumann conditions) (Figure 1.22) or in the presence of tertiary amines in organic solvents [41]. Z-Cl is commercially available and can also be prepared by the treatment of benzyl alcohol with phosgene (*caution*: phosgene is a highly poisonous gas and should be handled with extreme caution). However, the formation of Z-protected dipeptides as side-products and acylation of the hydroxy group of Ser and Thr and the phenolic function of Tyr (Z-Tyr-OH is obtained by alkaline hydrolysis of the corresponding bis-Z derivative) are the disadvantages of this highly active reagent. Hence, the moderately reactive mixed carbonate, Z-succinimido carbonate (Z-OSu) **39** is increasingly used in the preparation of Z-amino acids. Z-OSu furnishes good yields of Z-amino acids and also minimizes the formation of peptide impurities [42]. The reagent is commercially available and can also be prepared by the treatment of *N*-hydroxysuccinimide with Z-Cl. It is stable and can be stored without decomposition for a long time at low temperature with the exclusion of moisture. Most of the Z-amino acids are obtained as crystalline solids. The oily Z-amino acids can be crystallized as DCHA salts. Benzyl benzotriazolyl carbonate (Z-OBt) **41** and dibenzyldicarbonate (Z_2O or benzyl pyrocarbonate or Z-anhydride) **42** are the other Z-donors (Figure 1.23) proposed for the preparation of Z-amino acids [43, 44].

Cleavage The favored methods for the removal of N^{α}-Z groups are catalytic hydrogenation and acidolyis. Reagents and conditions, and common side-reactions encountered under each method of deblocking the Z group are furnished in Table 1.1.

The acid lability of Z group can be modulated by placing electron-withdrawing or -donating groups on the phenyl ring (Figure 1.24). When $X = NO_2$ (**43**), Cl (**44**), or Ph-N=N (**45**) (electron-withdrawing substituents), the acid stability of the groups increases due to the destabilization of the benzyl cation produced during acidolytic

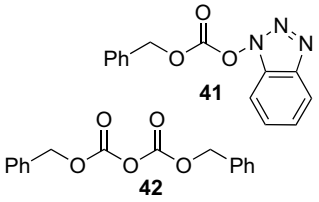
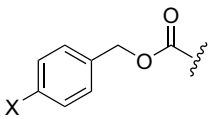


Figure 1.23 Z-donors.

Table 1.1 Reagents and conditions for the removal of N^{α}-Z protection.

Reaction with reagent and conditions	Notes
Catalytic hydrogenation employing H_2 in the presence of catalysts like Pd/C or Pd/$BaSO_4$ in MeOH, EtOH, or AcOH [45] (promoted by the addition of small amount of acids)	*N*-alkylated peptides can be formed due to a series of side-reactions starting from palladium-catalyzed oxidation of the alcohol (solvent) to aldehyde. The aldehyde forms Schiff's base with the deprotected amine, which in turn undergoes reduction to *N*-alkylated products. This can be avoided by carrying out hydrogenation in completely oxygen-free medium or with the addition of small amount of water (to suppress oxidation). Alcohols such as *iso*-propanol, which are resistant to oxidation, can be used as solvent.
	Not compatible with sulfur-containing amino acids due to catalytic poisoning
Catalytic hydrogenation employing Pd-$BaSO_4$ catalyst [45b]	Compatible with Met-containing peptides, but not with peptides with *S*-alkyl-Cys residues.
Catalytic hydrogenation in liquid ammonia solvent at $-33\,^{\circ}C$ [46]	Compatible with sulfur-containing amino acids including *S*-alkyl-Cys.
Silyl hydrides such as triethylsilane or *tert*-butyldimethylsilane in the presence of $PdCl_2$ [47]	
Sodium in liquid ammonia (Birch reduction)	
CTH with hydrogen donors such as 85% HCOOH, $HCOONH_4$, cyclohexenes, and hexadienes in the presence of Pd/C catalyst; $HCOONH_4$ and Pd/C under microwave irradiation in *iso*-propanol solvent [48]	The C^{α}–C^{β} double bond of didehydroalanine (ΔAla) residues is stable to CTH.
Acidolysis using anhydrous liquid HF, HBr-AcOH, pyridinium polyhydrogen fluoride (30% pyridine/70% HF), sulfonic acids such as methane sulfonic acids, fluoro- or trifluorosulfonic acid in DCM or TFA [49–51]	Benzylation of Tyr and Trp and *S*-benzylation of Met due to the formation of benzyl cation. This can be controlled by the addition of anisole or thioanisole as scavenger.



43: X=NO_2; **44**: X=Cl; **45**: X=Ph-N=N;
46: X=OMe; **47**: X=Me

Figure 1.24 Substituted Z groups.

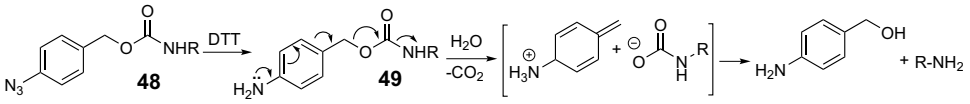


Figure 1.25 Cleavage of the Z(N_3) group via reduction of the azide.

cleavage [52–54]. The mechanism of acidolytic cleavage is shifted towards the S_N2 pathway by these substituents. The electron-releasing substituents (X = OMe (**46**) or Me (**47**)) increase the acid lability of the groups by offering higher resonance stabilization to the benzyl cation formed during cleavage [55, 56]. Deblocking preferentially occurs through an S_N1 pathway. In terms of hydrogenolytic cleavage of **43**, **45**, and **48**, a deviation from the standard mode of alkyl-oxygen bond fragmentation is observed. When X = N_3 (**48**), the dithiothreitol (DTT)-mediated reduction of the azido group results in the formation of 4-aminobenzyloxycarbonyl derivative **49** which undergoes a 1,6-electron electron shift to liberate the amine (Figure 1.25) [57]. Similarly, **43** and **45** undergo rapid hydrogenolysis even in neutral solution due to the formation of the same intermediate **49**. The substitutions also impart favorable properties such as a higher tendency to crystallization (**46**) and coloration (**44**) to the protecting group. The presence of a chromophore is helpful for monitoring reactions through spectrophotometric methods.

1.2.2.2.2 Urethanes Cleaved by β-Elimination

9-Fluorenylmethoxycarbonyl (Fmoc) Group [58, 59] The Fmoc group was introduced for peptide synthesis in the 1970s. The group is completely stable to acids and to a large extent to catalytic hydrogenation, although prolonged catalytic hydrogenation can cleave the group (this surprising reactivity of Fmoc to catalytic hydrogenation has been attributed to the β-phenylethyloxy skeleton that can be fragmented through hydrogenolysis, although much less readily than arylmethyloxy system). It is base-labile and removed by treatment with alkyl amines such as piperidine and diethylamine (DEA). The base-labile property of the Fmoc group introduced a "third dimension" to the then existing deprotection reactions, which mainly consisted of hydrogenolytic and acidolytic cleavage of benzyl- and *tert*-butyl-based protections, respectively. Further, the acid stability of the Fmoc group made possible the preparation of stable and highly active N^{α}-urethane-protected amino acid chlorides (the same are not accessible with Z- and Boc-protected amino acids) for rapid and difficult peptide couplings. Presently, Fmoc is a well-established and an extensively used α-amino protector for peptide synthesis, in general, and for solid-phase synthesis, in particular. A few prominent advantages of the group that have led to its popularity are:

i) The protection strategy based on the combination of Fmoc group (for N^{α} protection) and *tert*-butyl-based groups (carbamates, esters and ethers, for side-chain protection) – the Fmoc/*tert*-butyl approach – is superior to the traditional Boc/benzyl approach since the repetitive deblocking of the Fmoc group by base treatment does not cause a progressive loss of side-chain protections. It also enables the use of acid-labile linkers and resins for SPPS

from which peptides can be obtained as free acids directly. In contrast, the Boc/benzyl approach suffers from the partial loss of side-chain protections with every cycle of acidolytic removal of N^{α}-Boc group. It also precludes the use of acid-labile solid supports.

ii) The Fmoc group can be cleaved using mild reagents such as piperidine.

iii) With Fmoc as protective function, the progress of reactions can be monitored through UV (λ_{max}: 267, 290, 301 nm) and fluorescence spectrometry – a property that can be used to advantage in fully automated peptide synthesis.

- **Preparation:** 9-Fluorenylmethyl chloroformate (Fmoc-Cl) **51** and Fmoc-OSu **52** are the favored reagents for introducing the Fmoc group. Fmoc-Cl is commercially available (also prepared by the action of phosgene on 9-fluorenylmethanol) and storable for a long time under anhydrous conditions at low temperature. Acylation of amino acids with Fmoc-Cl is carried out under Schotten–Baumann conditions at 0 °C (Figure 1.26) [59]. Formation of detectable amounts of Fmoc protected peptide impurities is a major concern of this reagent, but this has been considerably overcome with modifications in the reaction conditions (see above). Fmoc-OSu is the reagent of choice for the preparation of Fmoc-amino acids [60]. It is commercially available, storable, and furnishes impressive yields without causing the formation of dipeptide side-products. The other reagents that have been proposed as Fmoc donors are the azido formate, Fmoc-N_3, and 9-fluorenylmethyl-1-chloroalkyl carbonate [61, 62].
- **Cleavage:** The Fmoc group is deblocked by nonhydrolytic base treatment by a variety of organic amines, but most efficiently by unhindered cyclic amines such as piperidine and morpholine in polar solvents such as DMF. The group is cleaved through an E_1C_B mechanism as shown in the Figure 1.21 (type B). The rate-limiting step is the abstraction of the proton from the bulky 9-fluorenylmethyl ring, and hence deblocking by sterically crowded amines is sluggish and often incomplete. The possibility of premature deblocking of the group during coupling due to the basicity of the free amino group of amino acid ester or peptide ester has been studied, and found to be at unalarming levels due to a huge difference in the rates of coupling and deblocking. However, when couplings are slow, as in the case of the synthesis of difficult sequences such as polyproline, deblocking of Fmoc can take place to a higher extent (also Pro is a secondary amine that can deblock Fmoc more effectively). Precautions such as slow addition of a solution

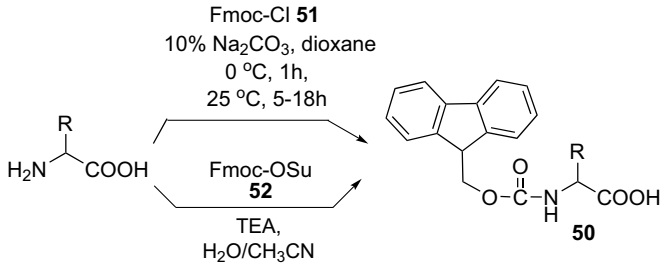


Figure 1.26 Preparation of N^{α}-Fmoc-protected amino acids.

of the free amino compound to a cold solution of activated Fmoc-amino acid (to create a high dilution of amine), and addition of acidic reagents such as HOBt and certain phenol derivatives (to bring down the basicity of the medium) can suppress a low level of deblocking that could happen during coupling. However, in SPPS the premature deblocking is unlikely due to spatial separation of the resin bound amines. Generally, the cleavage of a urethane-type protection (Boc and Z groups) results in the formation of byproducts (volatile or water-soluble compounds) that are easily separable On the other hand, fragmentation of Fmoc gives rise to dibenzofulvene (DBF), a reactive electrophilic reagent (Michael acceptor), as the byproduct. The presence of DBF is undesirable since it tends to polymerize, forming an insoluble mass or gels. It can also form an irreversible addition product with the deblocked amine. However, these potential problems are alleviated since the DBF is trapped by the deblocking amine, which is typically used in excess, through a facile formation of an adduct (a tertiary amine) that can be separated from the desired products. The adduct formation is dependent on factors such as the intrinsic basicity of the deblocking amine. For instance, amines such as piperidine, morpholine, and piperazine form adducts, while DEA does not. Further, the adduct formation is a reversible reaction and the position of equilibrium depends on the nature of the amine. Based on the criteria of the removal of unreacted deblocking amine, formation of DBF adduct, and the mode of its separation from products, different approaches have been developed for the selection of reagents for solid- and solution-phase cleavage of the Fmoc group. Such distinction is normally not made in the case of the other commonly used urethane protections (e.g., Boc group) where the reagents for solid- and solution-phase cleavage are essentially the same except that an excess amount is used on solid supports.

- **Deprotection in Solid Phase**: In SPPS, the process is straightforward without the need for special consideration for the removal of unreacted deblocking amine and the DBF adducts since these can be washed off the resin. The most widely used combinations are 20–50% piperidine in DMF or *N*-methyl-2-pyrrolidone (NMP) and 60% DEA in DMF [63–65].
- **Deprotection in Solution Phase**: The two main considerations are the separation of DBF adducts from the products and the complete elimination of the unreacted deblocking amine. These have been addressed by employing 4-(aminomethyl) piperidine (4-AMP) as deblocking as well as scavenging reagent [66]. 4-AMP forms the adduct which is soluble in phosphate buffer (pH 5.5) and hence can be removed by extraction with the buffer. The unreacted 4-AMP is removed prior to buffer wash by extraction with saturated NaCl solution. However, separation of the deblocked amine from 4-AMP-DBF adduct can sometimes be inefficient due to the formation of emulsions during extraction. This limitation has been overcome by replacing 4-AMP with tris-(2-aminoethyl)amine (TAEA) [67]. Both the amines have been employed in the Fmoc/polyamine approach to rapid peptide synthesis of several peptides including [Leu^5]enkephalin and substance P. A different approach to separate the adduct is to use polymer-supported amines such as polystyrene-bound or silica gel-bound piperazine as deblocking and scavenging reagents [68, 69]. The resin-bound DBF adduct and the unreacted

polymer-supported amine can be removed through filtration. However, complete scavenging has not been possible with these reagents with about 10–15% of residual DBF in solution. In this regard, it has been found that polymer-supported thiols are more efficient than polymer-supported amines as scavengers [70]. Alternatively, complete scavenging by polymer-supported amines can be achieved by substitution of indene based 3-indenylmethyloxycarbonyl (Imoc) and 2-chloro-3*H*-indenylmethyloxycarbonyl (Climoc) groups for Fmoc group as base-labile N^{α}-protectors [58]. Fmoc can also be deblocked using volatile amines such as DEA or dimethylamine (DMA), which can be completely removed by evaporation after the reaction [71, 72]. Deblocking has also been accomplished by using catalytic quantities (3 mol%) of the non-nucleophilic base 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in combination with a large excess of 1-ocatane-thiol in THF or polystyrene-supported *N*-(2-mercaptoethyl)amine in THF [70]. The thiols function as scavengers of DBF. Other bases employed for deblocking are hydrazine, hydroxylamine, and Tesser's base (30: 9: 1 dioxane/MeOH/4 N NaOH) [59]. Deblocking can be carried out in nonbasic conditions by using fluorine reagents such as 0.02 M tetrabutylammonium fluoride (TBAF·$3H_2O$)/DMF in MeOH and KF/18-crown-6 in the presence of scavengers (thiols) [73–75]. However, *O*-silyl protections are sensitive to fluoride ion treatment.

- **Side-Reactions During Cleavage:** The base used for deblocking Fmoc can catalyze intramolecular aminolysis reactions leading to the formation of side-products such as diketopiperazine (or 2,5-dioxopiperazine or piperazine-2,5-dione or DKP) and aspartimide (Figure 1.27). Free amino dipeptide alkyl esters **53**, particularly those which contain Pro or NMA at the N-terminus (since the configuration of peptide bond is *cis*), readily cyclize to DKPs **54** in basic media. Formation of DKPs can be minimized by adopting methods like fragment condensation with Fmoc-dipeptide acids or using esters such as benzyl or *tert*-butyl esters that have lower reactivity to aminolysis for protection of the carboxy terminus of peptides. The latter approach has been employed for the successful solution-phase synthesis of cyclosporine O – a peptide with several NMAs using Fmoc chemistry [76]. Employing fluoride reagents for deblocking Fmoc can also prevent DKP formation.

  Fmoc Analogs: The low solubility of Fmoc-protected derivatives in organic solvents is a limitation of the group. This has been addressed by the development of Fmoc analogs such as 2,7-di-*tert*-butyl-Fmoc group **55** whose derivatives are about 2 times more soluble due to hydrophobic alkyl substitutions on the fluorenyl ring [77] However, deblocking of **55** is about 4 times slower than Fmoc. The Sulfmoc group **56** is useful to improve the purity of synthetic peptides [78].

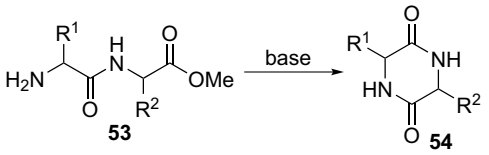


Figure 1.27 Base-catalyzed formation of DKP.

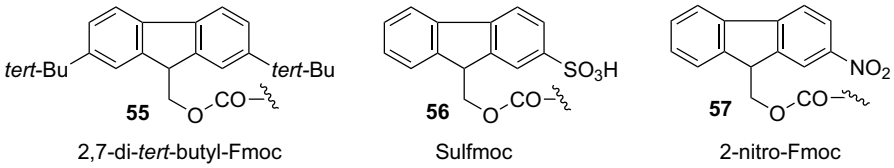


Figure 1.28 Fmoc analogs.

The strongly acidic Sulfmoc group can be introduced at the end of the synthesis to the growing peptide chain and the Sulfmoc-protected peptides can be efficiently separated from the rest of the non-growing and terminated peptides through chromatography. The 2-nitro-Fmoc group **57** is a photocleavable and optically active analog of Fmoc (Figure 1.28) [79].

Sulfonylethoxycarbonyl Groups The prototype 2-tosylethoxycarbonyl group (Tec) was introduced in 1964 [80] and since then a number of groups of the kind have been described. Presently, 2-(methylsulfonyl)ethoxycarbonyl (Msc) **58** and 2-(4-nitrophenylsulfonyl)ethoxycarbonyl (Nsc) **59** groups (Figure 1.29) are the important examples in the series. N^{α}-Msc-amino acids are prepared by using mixed carbonates Msc-OSu or Msc-ONp [81]. The group is stable to acids and to catalytic hydrogenation (an advantage over Fmoc) and does not inactivate the hydrogenation catalysts. Therefore, catalytic hydrogenation can be employed as a method of deblocking protecting groups from Msc-protected peptides. The group is cleaved within minutes in NaOH solution of pH 10–12 at 0 °C in the presence of MeOH, whose role is to trap the byproduct vinyl sulfone [82]. The reaction mixture should be acidified to decompose the stable carbamate intermediate, which may not be compatible with highly acid-sensitive groups. The Msc group is more hydrophilic and is favored when high solubility in aqueous media is desired. N^{α}-Nsc amino acids **60** are prepared through acylation of *N,O*-bis-TMS-amino acids with Nsc-Cl (Figure 1.30) [83]. The group can be an efficient substitute to Fmoc group due to: a higher tendency to crystallize, a

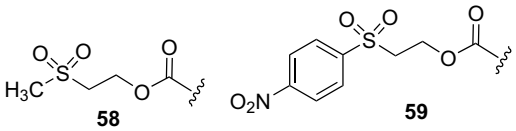


Figure 1.29 N^{α}-Sulfonylethoxycarbonyl groups.

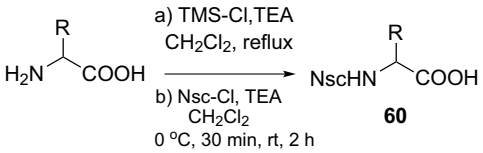


Figure 1.30 Preparation of N^{α}-Nsc-amino acids.

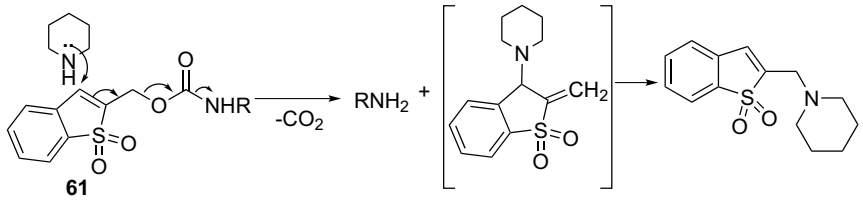


Figure 1.31 Mechanism of N^{α}-Bsmoc cleavage.

lower rate of cleavage (which leads to increased stability in DMF solvent and also avoids premature deblocking during coupling), the nonpolymerizing property of the vinyl sulfone byproduct, and less steric hindrance and lower rates of racemization of N^{α}-Nsc-protected His, Lys, and Ser.

1.2.2.2.3 **Urethanes Cleaved via Michael-Type Addition** A major concern with Fmoc is the reversible formation of DBF adduct and incomplete scavenging of DBF, particularly by polymer-supported amines. Consequently, groups such as 1,1-dioxobenzo[*b*]thiophen-2-ylmethoxycarbonyl (Bsmoc) **61**, 2-(*tert*-butylsulfonyl)-2-propenyloxycarbonyl (Bspoc), and 2-(methylsulfonyl)-3-phenyl-2-propenyloxycarbonyl (Mspoc) that are cleaved by a process in which the deblocking event (Michael-type addition) is simultaneously a scavenging event have been developed (Figure 1.31) [84]. The Bsmoc group additionally contains an alkyl substituent at the β-position of the Michael unit, which prevents premature deblocking of the group. The Bsmoc group is several times more sensitive to cyclic amines like piperidine than Fmoc group. It can be deblocked by piperidine solutions of lower concentration (2% v/v in DMF for 5 min as against 20% v/v in DMF for about 30 min for Fmoc removal), thus leading to minimum or no formation of side-products such as aspartimide that arise due to base-catalyzed reactions. In addition, the Bsmoc group can also be selectively cleaved over Fmoc group by using 2% TAEA in DCM. Further, when TAEA is used to deblock the Bsmoc group, a water-soluble adduct is formed that can be removed by extraction with water without the prerequisite for acidic phosphate buffer (as for Fmoc-TAEA adduct removal), which may cause partial loss of the deblocked amine. These advantages have amounted to the application of Bsmoc group in improved synthesis of several peptides including cyclosporine O [85].

A different category of base-labile *N*-protecting groups (e.g., 5-benzisoxazolylmethoxycarbonyl (Bic) group **62**) in which the deblocking is due to base-induced opening of the benzisoxazole ring followed by a 1,6-electron shift have also been proposed (Figure 1.32) [86].

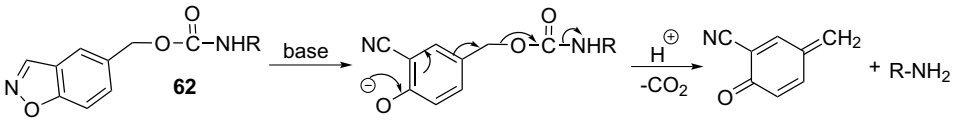


Figure 1.32 β-Elimination via isoxazole ring opening.

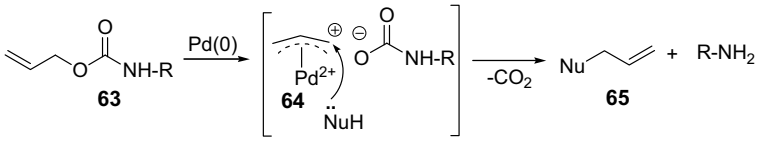


Figure 1.33 Pd(0)-mediated cleavage of allyl urethanes.

1.2.2.2.4 **Allyloxycarbonyl (Aloc) Group** Allyl-based protections were not paid much attention until 1987, when it was demonstrated that they could be selectively removed via a Pd(0)-catalyzed allyl transfer reaction (Tsuji–Trost reaction) under mild conditions [87]. Indeed, the discovery of this new method introduced what is now referred to as a "fourth dimension" to the protection schemes due to its compatibility with benzyl-based (removed by hydrogenolysis), *tert*-butyl-based (removed by acids), and 9-fluorenylmethyl-based (removed by bases) protections.

The Aloc group **63** is introduced using allyl chloroformate (Aloc-Cl) or diallyl dicarbonate $(Aloc)_2O$ [88, 89].

Cleavage The Aloc group is fragmented in the presence of a Pd(0) catalyst through the formation of η^3-allyl palladium complex **64**, which is subsequently decomposed by the transfer of the allyl group to a nucleophile (Figure 1.33). Allyl transfer to the deblocked amine is avoided by the addition of scavengers, which preferentially accept the allyl group from the palladium complex.

As catalyst, the commercially available *tetrakis*(triphenylphosphine)palladium(0) $[Pd(PPh_3)_4]$ is extensively used although palladium complexes with differentially substituted ligands have also been proposed. The reagents that can be employed as scavengers are summarized in Table 1.2.

1.2.2.3 Urethane Groups Derived from Secondary Alcohols

These groups (Figure 1.34) do not exhibit any unique properties complementary to the other routinely used N^{α}-amino protecting groups. Nonetheless, groups such as cyclopentyloxycarbonyl (Cpoc) **66**, 2-adamantyloxycarbonyl (Adoc) **67**, and isobornyloxycarbonyl (Iboc) **68** [94–96], which exhibit cleavage characteristics similar to the Boc group, have been used with limited scope. The di-2-pyridinylmethyloxycarbonyl (Dpoc) **69** group is fragmented by Zn-AcOH treatment similar to the Z group [97].

1.2.2.4 Urethanes Derived from Tertiary Alcohols

1.2.2.4.1 ***tert*-Butoxycarbonyl (Boc) Group** The Boc group was introduced in 1957 for peptide synthesis [98]. It was the first successful urethane-type protection that was orthogonal to the Z group, which was the only extensively used urethane protection then, due to its stability to catalytic hydrogenation (to which Z is labile) and its lability to mild acidic conditions (to which Z is stable). The group is resistant to alkali and to reduction by Na-liquid NH_3. Soon, it became an important amino protecting group to be used along with Z group. The introduction of the Boc group also made it possible to devise a general protection scheme based on *tert*-butyl/benzyl protections (for N^{α}

Table 1.2 Reagents and conditions for cleavage of N^{α}-Aloc protection.

Scavengers	Notes
Secondary amines Piperidine, DEA, morpholine;[a] 4-methylmorpholine in DCM/AcOH [90]	Excess nucleophile is required to displace the equilibrium of the allyl transfer reaction towards deprotection.
1,3-Dicarbonyl compounds as nucleophiles;[b] Dimedone, *N,N'*-dimethylbarbituric acid [91]	The liberated amine abstracts a proton from the acidic dicarbonyl compound, thus forming an ammonium derivative that is not allylated.
Hydride donors LAH, $NaBH_4$, phenylsilane ($PhSiH_3$), tributyltin hydride (Bu_3SnH), $BH_3{\cdot}NH_3$, $BH_3{\cdot}NHMe_2$ [92, 93]	The fragmentation results in a stable pseudometallic carbamate that is decomposed to the free amine through hydrolysis or acidolysis.
	If deprotection is carried out in the presence of an acylating agent, a transacylation can take place between the pseudometallic carbamate and the acylating agent leading a new amide bond. Hence, a tandem deprotection–coupling (with acid fluorides or active esters) of N^{α}-Aloc amino acids gives rise to peptides under almost neutral conditions.

a) Not compatible with Fmoc.
b) Compatible with Fmoc.

and side-chain functions), which was a major breakthrough for the expansion of SPPS methodology. Presently, the Boc group is the most favored N^{α}-protecting group (similar to Z and Fmoc) for both solution-phase synthesis and SPPS.

Preparation di-*tert*-Butyldicarbonate (di-*tert*-butylpyrocarbonate or $(Boc)_2O$) **71** and 2-(*tert*-butoxycarbonyloxyimino)-2-phenylacetonitrile (Boc-ON) **72** are the widely employed reagents for the preparation of Boc-amino acids **70** (Figure 1.35). $(Boc)_2O$ furnishes impressive yields in short duration and even at low temperature. In addition, the byproducts, CO_2 and *tert*-butanol, can be removed easily. The reagent is commercially available, stable, and can be stored for a long time at low temperature and under anhydrous conditions. It has also been used to prepare several *N*-Boc-protected unnatural amino acids [99]. Boc-ON provides good yields of Boc-amino acids [100] and is more soluble in organic solvents. However, the oxime byproduct is water insoluble and is removed by extraction with Et_2O. It is commer-

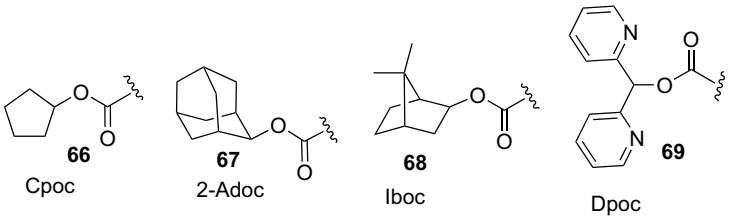


Figure 1.34 Urethane protections derived from secondary alcohols.

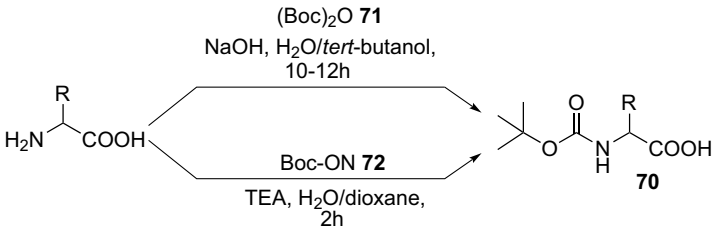


Figure 1.35 Preparation of N^{α}-Boc-amino acids.

cially available as well as stable for long duration. Acidification of the alkali salt of Boc-amino acids (and work up of *N*-Boc-protected compounds) is best done using 10% citric acid solution. Acids like HCl are avoided for this purpose due to the risk of partial cleavage of the group. The other important Boc donors proposed are Boc-F, Boc-N_3, and the mixed carbonates (*tert*-butoxycarbonyl succinimido carbonate (Boc-OSu) and *tert*-butoxycarbonyl-4-nitrophenyl carbonate (Boc-ONp)), and *N*-Boc-substituted heterocycles like Boc-imidazole [101]. With Boc-N_3 (prepared freshly from Boc-NH-NH_2), best yields are obtained by conducting the reaction in a pH stat.

Deprotection A wide range of reagents encompassing protic acids, Lewis acids, organosilanes, and even hot water (deionized water, Millipore grade) [102] have been proposed for deblocking the Boc group.

Protic Acids HCl in organic solvents and TFA are the most frequently employed reagents for deblocking N^{α}-Boc function. Presently, TFA is being increasingly favored. With both reagents, the cleavage proceeds through a S_N1 pathway with the formation of the *tert*-butyl cation, a strong alkylating agent that can cause the attachment of the *tert*-butyl group to nucleophilic sites of His, Met, Tyr, and Cys. The fate of the cation depends on the strength of the conjugate base of the deblocking acid. Since Cl^- is a weaker nucleophile the cation is trapped slowly into *tert*-butyl chloride and, in the process, a portion of the cation rearranges into unreactive isobutene. With TFA, the cation is completely trapped by the more nucleophilic trifluoroacetate ion to form *tert*-butyl trifluoroacetate, which is also a good alkylating agent. Also, since TFA is not strong enough to protonate nucleophilic centers of Met, Tyr, and Cys, these sites are exposed to alkylation. The addition of scavengers such as anisole or thioanisole or thiols can minimize the undesired alkylations. When thioanisole is added, deprotection takes place through a S_N2 mechanism with direct formation of the *tert*-butyl derivative of thioanisole. Hence no potential alkylating agents are formed. Deblocking in solution as well as on solid supports is carried out by using neat TFA or TFA in CH_2Cl_2 in the presence of a variety of scavengers [103]. A mixture of TFA and phenol/*p*-cresol and TFA in H_2O/DCM/AcOH can selectively remove the Boc group in the presence of acid sensitive *O*-silyl protections such as *tert*-butyldimethylsilyl (TBDMS) [104]. Trifluoromethane sulfonic acid (TfOH), methanesulfonic acid, anhydrous liquid HF, HBr in AcOH, and 98% formic acid are the other reagents used for cleavage. Anhydrous TsOH (2.0 M) in dioxane solution removes the Boc group without decomposition of Trp.

Organosilanes Progressive loss of benzyl-based protections and the peptide chains anchored to resins through benzyl ester linkages with each acidic deprotection cycle of TFA-mediated Boc cleavage is a major concern in the synthesis of long peptides through the Boc/benzyl protection strategy in SPPS. Cleavage of peptides from resin has been found to be about 0.7–2% per cycle. The loss of protections can be minimized by the use of organosilicon reagents such as TMS-Cl as highly selective reagents for Boc removal. A combination of 1 M TMS-Cl and 3 M phenol in DCM provides excellent selectivity along with quantitative cleavage [105]. With this reagent the loss of peptide chains has been drastically brought down to nearly 0.0004%, and that of benzyl ester, ether, and carbonate groups to about 0.01 to 0.17% for each per hour deprotection cycle. A variation of this method in which the silicon reagent is $SiCl_4$ has also been proposed [106]. Trimethylsilyl trifluoromethanesulfonate (TMS-triflate or TMS-OTf)/2,6-lutidine can selectively remove the Boc group on TFA-sensitive resins [107].

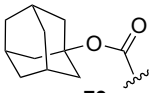
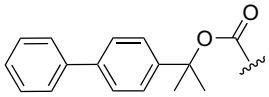
Lewis Acids Nonprotic acids such as $AlCl_3$, $BF_3{\cdot}OEt_2$ [108, 109], montmorillonite (acidic clay), celite, silica gel, and ion-exchange resins like Amberlyst 15 can also be used for deblocking the Boc group [110]. The last example can be used for simultaneous deprotection and purification of Boc-protected peptides. $AlCl_3$-mediated cleavage can be carried out in short duration under microwave irradiation [111]. When nonprotic acids are used for cleavage, the free amino peptide can be converted into its salt by the addition of HCl or HBr solutions in anhydrous Et_2O/EtOAc/dioxane to facilitate isolation, given there is no other acid-labile group.

Oxidizing Agent Excess ceric ammonium nitrate (CAN) in refluxing acetonitrile removes the Boc group [112]. The method is compatible with acid-sensitive TBDMS protections since the reaction is carried out under neutral conditions.

Removal of Boc Groups in the presence of tert-Butyl Esters Selective removal of Boc groups in the presence of acid-labile *tert*-butyl esters is of enormous practical utility since the *tert*-butyl esters are extensively used carboxy protections for peptide synthesis in solution. This has been accomplished using HCl/dioxane or 1.0 M HCl in EtOAc or concentrated H_2SO_4 (1.5–3.0 equiv.) in *tert*-butyl acetate or methanesulfonic acid (1.5–3.0 equiv.) in *tert*-butyl acetate/DCM (4: 1, v/v) [113]. The latter combination is based on the observation that *tert*-butyl carbamates are cleaved through an irreversible process with permanent loss of CO_2, while the cleavage of *tert*-butyl esters is reversible. Hence, addition of *tert*-butyl acetate as a source of *tert*-butyl cation shifts the equilibrium in the direction of acid-catalyzed esterification. TsOH (1.0 equiv.) in toluene [113] and Zeo-Carb 225/H^+ ion exchange resin have also been used to remove Boc groups with retention of *tert*-butyl-esters [114].

1.2.2.4.2 **Boc Analogs** Selected Boc analogs (**73**, **74**) with distinctive properties are listed in Table 1.3.

Table 1.3 Boc analogs.

Analogs of Boc	Notes
Adamantyl-1-oxycarbonyl (Adoc)	Cleavage sensitivity is similar to Boc; higher solubility and stability than Boc due to bulky hydrophobic group [115]
 **73**	
2-(Biphenyl-4-yl)-2-propoxycarbonyl (Bpoc)	Highly acid sensitive; removed under very mild acidic conditions with selectivity over Boc, Trt, acid-sensitive resin, and even extremely acid-sensitive thioxopeptides [116]
 **74**	Free acids are not stable to storage due to autocatalyzed acidolysis; storable as stable DCHA or CHA salts as well as stable active esters

1.2.2.5 Other Aspects of Urethane Protectors

1.2.2.5.1 Formation of Dipeptide Impurities during the Introduction of Urethanes and Protocols to Overcome It Formation of detectable amounts of *N*-protected dipeptide acids (and even tripeptide acids) has been observed (a solvent system of toluene/AcOH (10: 1) efficiently differentiates Fmoc-peptide acids from Fmoc-amino acids on thin-layer chromatography (TLC)) when chloroformates such as Fmoc-Cl and Z-Cl are employed for the preparation of N^{α}-urethane-protected amino acids. This has been explained based on the activation of the carboxy group of amino acids by chloroformate to mixed anhydrides followed by their aminolysis by the amino acids (Figure 1.36). The mixed anhydride intermediates are sufficiently stable in aqueous/organic reaction mixture and give rise to peptides even under Schotten–Baumann conditions. The peptide acids are difficult to separate from the products through work-up or crystallization or column purification. As a result, their presence decreases the homogeneity of *N*-protected amino acids, which are the building blocks for the synthesis of products (peptides) that are expected to be of highest purity. This side-reaction is also a major concern for large-scale and industrial production of *N*-protected amino acids.

Certain modifications in the reaction conditions have been proposed for dipeptide-free synthesis when Fmoc-Cl is used for the introduction of Fmoc group. A combination of 20–25% excess of amino acid over Fmoc-Cl, a 4-fold excess of Na_2CO_3 over amino acid, and 1: 10 (v/v) mixture of dioxane and water as the solvent system has been recommended to be suitable [117]. However, the method is not appealing in the case of sterically hindered amino acids, Val and Leu. Consequently, performing the acylation in neutral conditions has been demonstrated to be an

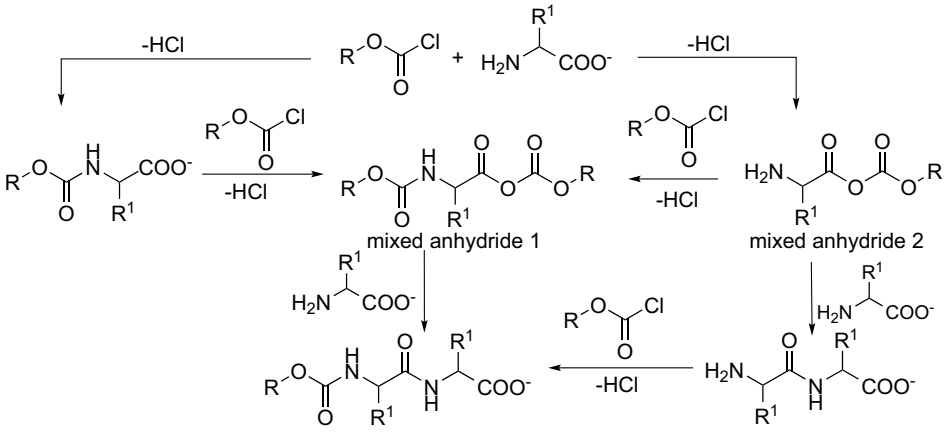
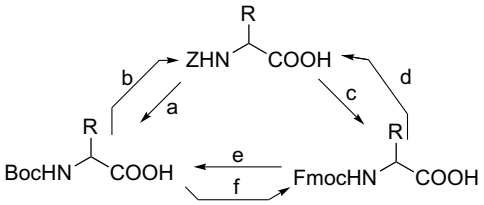


Figure 1.36 Reaction mechanism for the formation of oligopeptides during introduction of N^{α}-urethane protections.

efficient alternative. In a basic medium, the carboxy group exists as the carboxylate ion, which reacts with the chloroformate and forms a mixed anhydride. The usage of nonbasic reagents like zinc dust to eliminate HCl (liberated during acylation of the α-amino group by Fmoc-Cl) without raising the pH of the medium, prevents ionization of the carboxy group and in turn, the formation of mixed anhydrides. Thus, the possibility of the formation of a dipeptide is eliminated [118]. Acylation of *N,O*-bis-TMS-amino acids also avoids the formation of mixed anhydrides since the carboxy group in these compounds is protected as a silyl ester [119].

Dipeptide formation can also be avoided by substituting moderately reactive Fmoc-OSu and Fmoc-N_3 for highly active Fmoc-Cl as Fmoc donors. In these cases, only the α-amino group, which is more nucleophilic than the carboxy group, reacts with the reagents. However, the acylation can be comparatively sluggish and lower yielding particularly with Fmoc-N_3. Fmoc-OSu has been the reagent of choice and the best alternative to Fmoc-Cl for the preparation of N^{α}-Fmoc-amino acids for a long period. Rigorous high-performance liquid chromatography (HPLC) analysis has recently shown that the peptides synthesized using Fmoc-amino acids are contaminated with Fmoc-β-Ala-OH and Fmoc-β-Ala–Xaa-OH to the extent of 0.1–0.4%. The origin of these impurities has been explained based on a process that involves nucleophilic ring opening of the HOSu moiety of Fmoc-OSu and Lossen rearrangement of the resultant *O*-acyl hydroxamate intermediate as key reactions [120]. Consequently, Fmoc-2-mercaptobenzothiazole (MBT) has been proposed as an alternative to Fmoc-OSu [120]. This new Fmoc donor has shown to cause no formation of dipeptide as well as β-Ala impurities. The reagent can be prepared by the treatment of the DCHA salt of MBT with Fmoc-Cl in chloroform.

1.2.2.5.2 **Introduction of Urethanes via Transprotection** Interchange of N^{α} protections is relevant when a switch over of the chemoselectivity of protecting groups is required due to a change in the synthetic methods. In this regard, benzyl,



(a) cyclohexadiene, 10% Pd/C, $(Boc)_2O$, EtOH, rt; (b) TFA, DCM, 1h, 0 °C, Z-Cl, aq. Na_2CO_3, Dioxane; (c) H_2, 10% Pd/C, 2, 2′-dipyridyl, Fmoc-OSu, MeOH; (d) KF, TEA, DMF, benzyl- 5-norbornene-2,3-dicarboximido carbonate (Z-ONdc), rt; (e) KF, TEA, $(Boc)_2O$; (f) aqueous HCl, Fmoc-OSu, NaOH, rt, 6 h

Figure 1.37 Interconversion of N^{α}-urethane protections.

9-fluorenylmethyl and *tert*-butyl carbamates have been successfully interconverted (Figure 1.37) [121].

1.2.2.5.3 **Protection of the Nitrogen of α-Amino Acid *N*-Carboxy Anhydrides (NCAs) 122** *N*-Carboxy anhydrides (NCAs) **75** (Leuchs anhydrides or internal anhydrides of amino acids) represent simultaneously protected and activated amino acid derivatives. However, polymerization and rapid hydrolysis properties of NCAs make acylations involving them difficult. Nonetheless, protection of the nitrogen of NCAs converts them into efficient peptide coupling agents. Urethane-protected NCAs **76–78** (urethane-protected *N*-carboxy anhydrideUNCAs) are particularly advantageous since they retain the reactivity of NCAs, but exclude the possibility of oligomerization. In addition, they are stable, and can be crystallized and stored. Fmoc- and Z-protected NCAs are prepared by treating NCAs with the corresponding chloroformates (Figure 1.38). *N*-Methylmorpholine (NMM) is specifically used as base in this reaction since unlike most of the tertiary amines, it does not catalyze polymerization of the NCAs. Boc-NCAs **78** are prepared by using Boc_2O. On the other hand, Trt-protected NCAs **79** can be obtained directly from N^{α}-Trt-amino acids by treatment with triphosgene [123].

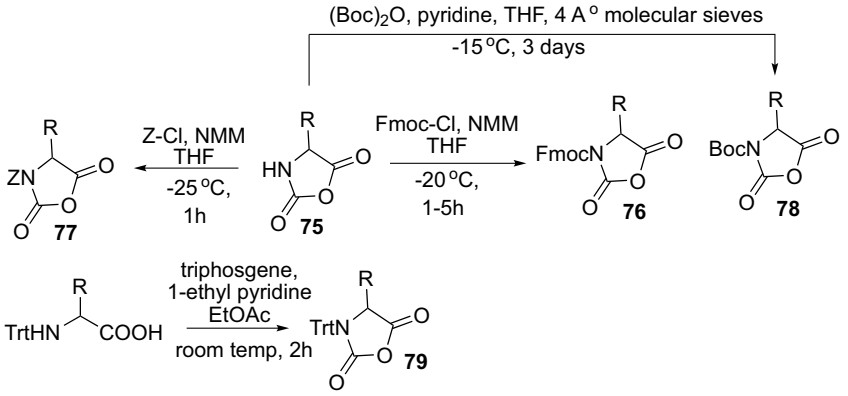
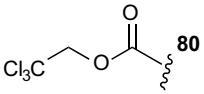


Figure 1.38 Protection of NCAs.

1.2.2.5.4 **N^{α},N^{α}-bis-Protected Amino Acids** Double protection of the nitrogen of the α-amino group is occasionally required to avoid side-reactions arising from the acidic -NH proton of the urethane linkage of N^{α}-protected amino acids/peptides. The N^{α},N^{α}-bis-Boc protection is introduced by the treatment of benzyl esters of Boc-amino acids with $(Boc)_2O$ in the presence of 4-(dimethylamino)pyridine (DMAP) in CH_3CN, followed by catalytic hydrogenation [124, 125]. The product is purified from the possible *N*-mono-Boc-contaminants by recrystallization in petroleum ether. N^{α}-Z,N^{α}-Boc-amino acids are prepared by treatment of 9-fluorenylmethyl esters of Z-amino acids with $(Boc)_2O$ in the presence of DMAP followed by cleavage of the ester with piperidine [125]. The N^{α},N^{α}-bis protected derivatives can be synthetically valuable compounds. The N^{α},N^{α}-bis-Boc-amino acids as well as N^{α},N^{α}-Boc, Z-amino acids can be converted into Boc- and Z-protected NCAs, respectively by the action of $SOCl_2$ in DMF [126]. This route presents an alternative to the preparation of UNCAs since it obviates the requirement of a NCA precursor. The bis-Boc derivatives can be transformed to mono Boc derivatives by selective elimination of one Boc moiety by treatment with hydrazine or LiOH or 20% magnesium perchlorate [127]. Under standard conditions of peptide coupling, the activated N^{α},N^{α}-bis-alkyloxycarbonyl-amino acids do not racemize via the formation of oxazol-5-one intermediates. However, enolization through base-catalyzed α-proton exchange (leading to racemization) can be promoted by the presence of an additional electron-withdrawing substituent on the α-amino group. This can be minimized through rapid couplings by using highly active Boc_2-amino acid fluorides as acylating agents [126, 128]. However, N^{α},N^{α}-bis-Boc protected dipeptides with C-terminal Gly show an increased tendency to form hydantoins rather than the corresponding Boc-dipeptides.

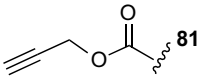
1.2.3
Other N^{α}-Protecting Groups

2,2,2-Trichloro-ethyloxycarbonyl (Troc)



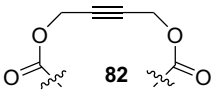
Cleaved using zinc or cadmium or their couples. Fission of alkyl-oxygen bond is thorough Grob fragmentation [129].

Propargyloxycarbonyl (Poc)



Stable to acids and bases. Removed using tetrathiomolybdate salt [130].

Bis-but-2-ynyloxycarbonyl (Bbc)



C_2 symmetric protecting group, introduced by treating amino acid methyl esters (2 equiv.) with Bbc-Cl followed by ester hydrolysis. The group is stable to acids (6 N HCl, 12 h) and bases (4 N NaOH, 12 h), removed using tetrathiomolybdate salt as well as resin-bound tetrathiomolybdate [131].

1.2.3.1 α-Azido Acids as α-Amino Acid Precursors

The conversion of the α-amino group into an azido group eliminates the problem of competing nucleophilicity. This transformation can be easily reversed to regenerate the α-amino group through reduction of the azide. α-Azido acids can be synthesized by azidolysis of α-bromo acids (synthesized by diazotization of amino acids in the presence of KBr or α-bromination of alkyl acids by *N*-bromosuccinimide (NBS)) with NaN_3 [132]. Alternatively, optically pure α-azido acids can be obtained in a single step by treating amino acids with triflyl azide [133]. α-Azido acids can be converted into α-azido acid bromides and chlorides (acid bromides of N^{α}-urethane-protected amino acids are not accessible; but N^{α}-Phth- and Nbs-amino acids can be converted to acid bromides), which are particularly useful for difficult couplings. They are very reactive, do not racemize (since they are unable to form oxazol-5-ones), and offer no steric hindrance to coupling [134]. Catalytic hydrogenation or treatment with phosphine followed by hydrolysis coverts the α-azido group into an α-amino group [135]. The azido compounds can be directly converted into a carbamate through reduction in the presence of a urethane donor [136]. *In situ* condensation into peptides is also possible through treatment with *tert*-butylphosphine in the presence of phenyl diselenide and an N^{α}-protected amino acid [137].

1.2.3.2 One-Pot N^{α} Protection and C^{α} Activation

The two key reactions in peptide synthesis, N^{α} protection and α-carboxy activation, can be executed in a single pot when pentafluorophenyl carbonates such as 9-fluorenylmethylpentafluorophenylcarboante (Fmoc-OPfp) are employed as urethane donors. N^{α}-Fmoc-amino acid pentafluorophenyl esters can be directly obtained by *in situ* esterification of the pentafluorophenol (liberated during acylation of the α-amino group by Fmoc-OPfp) by the addition of *N,N'*-dicyclohexylcarbodiimide (DCC) to the reaction mixture after the formation of N^{α}-Fmoc-amino acids [138]. This approach has been extended to prepare a variety of *N*-urethane-protected amino acid Pfp esters by treating amino acids with 2 equiv. of pentafluorophenyl carbonates (which display a dual role as urethane donors as well as carboxy activators). A series of Pg-Xaa-OPfp, where Pg = Aloc, Z, Boc, or Troc, has been synthesized via this method [139].

1.2.3.3 Effect of N^{α}-Protecting Groups in the Synthesis of NMAs

The two important approaches to synthesize NMAs are methylation of N^{α}-protected amino acids/esters and the reductive ring opening of oxazolidin-5-ones derived from *N*-protected amino acids [140]. The feasibility of the former approach is strongly dependent on the nature of N^{α} protection. N^{α}-Boc- and Z-amino acids are treated with a strong base such as NaH or KOH (finely powdered solid) to remove the NH proton of the carbamate followed by methylation using MeI or Me_2SO_4. The strongly basic condition is not compatible with substrates bearing the Fmoc moiety. An alternative and mild method is to use Ag_2O/MeI, but the product obtained will be in the form of a methyl ester, which is readily racemized during alkaline hydrolysis *en route* to its conversion into a free acid. On the other hand, N^{α}-sulfonamide-protected amino acids can be methylated using mild and racemization free conditions without the requirement of a strong base due to the increased acidity of the sulfonamide

nitrogen. N^{α}-Nosyl- as well as N^{α}-Nbs-protected amino acids are treated with methylating agents such as methyl *p*-nitrobenzyl sulfate in the presence of bases like 7-methyl-1,5,7-triaza-bicyclo[4.4.0]dec-5-ene (MTBD) or K_2CO_3 to obtain the corresponding NMAs. An even milder method is to use diazomethane as the methylating agent under neutral conditions. Site-selective methylation of the sulfonamide nitrogen of N^{α}-sulfonyl-protected peptide or an orthogonally protected amino acid derivative is also possible due to the substantial difference in the acidity of NH protons of the sulfonamide unit and peptide or carbamate bonds. Reduction of oxazolidin-5-ones obtained from N^{α}-protected amino acids by silyl hydrides in presence of TFA furnishes good yields of NMAs and is compatible with most of the urethane protections. It is especially useful for the synthesis of N^{α}-Fmoc-protected NMAs. The catalytic hydrogenation of oxazolidin-5-ones of Z-amino acids has been shown to directly give free amino NMAs [140b]. However, the usefulness of this route as an efficient means to access NMAs has not been undoubtedly established (elimination of formyl carbon from oxazolidinone upon removal of the *N* protection, thereby giving rise to amino acids has been observed in several cases).

1.3 Carboxy Protection

Protection of the carboxy group is not mandatory if carboxy activation and peptide couplings are carried out in separate steps (e.g., active ester coupling method). α-Amino acids can be acylated exclusively at the amino group since it is a much stronger nucleophile than the carboxy group. However, for couplings carried out via *in situ* activation of the carboxy group in the presence of the amino component (e.g., carbodiimide-mediated couplings), the carboxy group of the amino acid that is acylated has to be protected to avoid its self-condensation. However, irrespective of the kind of chemistry used for peptide bond formation, it has become a common practice to protect the carboxy group as part of a global protection strategy. The advantages associated with using carboxy-protected substrates for synthesis are: improved solubility in organic solvents, ease of purification and product isolation, suppression of the acidity of substrates, and prevention of side-reactions including racemization arising from the reactivity of the free carboxy group.

Since the carboxy protection is a semipermanent protection, it is expected to survive the conditions of repetitive deblocking of α-amino protections. The most important and extensively used method for carboxy group protection is esterification. Conversion into amides is another possibility, but this is not adopted as the amides represent irreversible protection due to the lack of methods for their selective hydrolysis in the presence of the peptide bond. Esters are cleaved via the fission of acyl-oxygen or alkyl-oxygen bonds. The cleavage parameters are determined by the structure of the alcohol component of the ester. Esters and carbamates which are derived from the same alcohol are cleaved via a fundamentally similar mechanism. Hence, there exists a colinear relationship between an ester–carbamate pair with respect to cleavage conditions. Boc and *tert*-butyl esters (cleaved by acids), Z and

benzyl esters (cleaved by hydrogenolysis), Fmoc and Fm esters (cleaved by base), and Aloc group and allyl esters (cleaved by Pd(0)-catalyzed allyl transfer) are the important examples for such a relationship.

1.3.1 Methyl and Ethyl Esters

There is essentially no difference between the unsubstituted methyl and ethyl esters in terms of methods of introduction as well as cleavage, although the latter are more resistant to attack by base and nucleophile. Methyl and ethyl esters are prepared by acid-mediated esterification of amino acids with the corresponding alcohol. Originally, anhydrous HCl was used in the preparation. A more efficient and milder protocol is to use $SOCl_2$ wherein the reaction can be carried out at 0 °C and the gaseous byproducts, HCl and SO_2, can be easily removed [141]. Amino acid methyl esters are isolated and stored as HCl salts, but have to be deprotonated to the free amino methyl esters for their utilization as amino components in coupling. The salts are generally deprotonated *in situ* by the addition of an equimolar quantity of tertiary amine followed by treatment with the acid component for peptide coupling. Nonetheless, this regular practice is associated with shortcomings such as difficulty in the addition of a stoichiometric amount of the tertiary amine, which may result in transfer of excess of base. The presence of excess base can promote side-reactions such as O-acylations and aspartimide formation, and racemization of the carboxy-activated residues. The rates of these reactions are influenced by the basicity of the tertiary amine. Using NMM as a preferred base for deprotonation can minimize these side-reactions. On the other hand, amino acid methyl esters can be obtained (whenever needed) by treating the suspension of the hydrochloride salt in organic solvent with aqueous $NaHCO_3$ or an equivalent amount of a tertiary amine (usually TEA or NMM) followed by extraction into EtOAc or Et_2O. The use of aqueous $NaHCO_3$ for deprotonation, although it eliminates side-reactions, can lead to reduction in the yields of amino acid methyl esters during extraction into the organic layer. Alternatively, deprotonation can be carried out in a base-free medium by using activated zinc dust as the proton scavenger. After deprotonation, the excess zinc and $ZnCl_2$ are removed by filtration, and the organic solvent is evaporated to obtain amino acid methyl esters in quantitative yields [142]. The acidic condition of preparation may render peptide and acid-sensitive protections unstable. Hence, methyl esters of N^{α}-protected (and side-chain protected) amino acids and peptides are prepared by using diazomethane (ethereal solution at 0 °C) – a mild and efficient methylating agent [143]. Diazo(trimethylsilyl)methane is a safer alternative to diazomethane (toxic and explosive).

Methyl as well as ethyl esters are stable to acidolysis (HBr in AcOH, TFA), catalytic hydrogenation, and to nucleophiles such as thiols and amines. They are cleaved by saponification with methanolic or ethanolic KOH or NaOH or LiOH in a wide range of solvents such as DMF, dimethyl sulfoxide (DMSO), pyridine, and aqueous mixtures of alcohol, acetone, EtOAc, and dioxane [144]. Methyl esters of small peptides are hydrolyzed within 0.5–1.5 h at room temperature, but hydrolysis

becomes increasingly difficult with the length of the peptide chain. Methyl esters of long-chain peptides require excess alkali and longer reaction times and sometimes elevated temperatures for completion of cleavage. The Fmoc group is not stable under the standard conditions of methyl ester cleavage. Nonetheless, addition of 0.8 mmol of $CaCl_2$ has been shown to increase the lifetime of Fmoc protections in alkaline hydrolytic conditions (1.2 equiv. of NaOH in *iso*-propanol/water mixture at 20 °C) [145]. Two equivalents of LiOH in THF at 0 °C has also been found to cause selective cleavage of methyl ester in the presence of N^{α}-Fmoc function [146]. The base used for methyl ester cleavage can promote racemization (either by base-catalyzed α-hydrogen exchange or 5(4*H*)-oxazolone formation) of the C-terminal amino acid (which is more pronounced in the case of peptides containing NMAs and Cys(Bzl) residues), DKP formation, and hydantoins from Z-Xaa–Gly-OMe peptides. Alkaline treatment can also promote the formation of ΔAla from hydroxy protected Ser residues. In this regard, tetrabutylammonium hydroxide (TBAH) has been proposed as the reagent that cleaves methyl esters best without causing racemization [147]. Trimethylsilyliodide (TMS-I) and bis-(tributyltin) oxide (BBTO) can be used as nonbasic reagents to circumvent many of the base-catalyzed side-reactions [148].

1.3.1.1 Substituted Methyl and Ethyl Esters

These esters (**83–90**) are cleaved under different conditions than the simple methyl or alkyl esters. Selected examples are shown in Table 1.4.

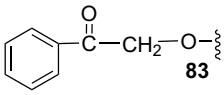
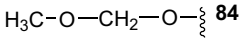
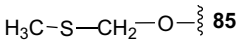
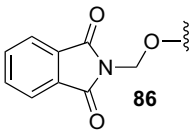
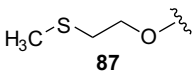
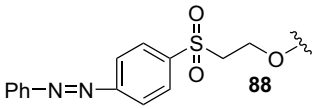
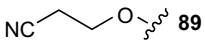
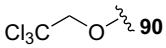
1.3.2 Benzyl Ester

Benzyl esters were introduced to peptide synthesis [157] along with the Z group, both of which are cleaved by catalytic hydrogenation and acidolysis, although the ester is comparatively less reactive to the latter reaction. Benzyl esters are stable to moderate basic conditions and to nucleophiles. Amino acid benzyl esters are typically prepared by esterification with benzyl alcohol in the presence of TosOH in benzene or toluene under azeotropic removal of water in a Dean–Stark apparatus [158]. This esterification has been efficiently carried out within minutes under microwave irradiation [159]. Dibenzyl esters of Asp and Glu can also be prepared by the same protocol and the ω-benzyl ester can be selectively cleaved by HI to obtain α-carboxy monobenzyl ester [160]. Benzyl esters of amino acids are obtained as TosOH salts which can be stored under anhydrous conditions. They are deprotonated using procedures similar to that of methyl esters. N^{α}-Protected amino acid benzyl esters can be prepared by the treatment of cesium salt of the corresponding amino acids with benzyl bromide [161] or by DCC-mediated esterification with benzyl alcohol in the presence of DMAP catalyst [162].

1.3.2.1 Cleavage

Quantitative cleavage is accomplished through catalytic hydrogenation in solvents such as alcohols and dioxane usually within 1 h for small peptides, but prolonged duration is required for long-chain peptides. Benzyl esters of sulfur-containing

Table 1.4 Substituted methyl and ethyl esters.

Ester	Cleavage conditions
Phenacyl (Pac)[a)]  **83**	Zn/AcOH at room temperature, NaSPh in DMF, TBAF in DMF at room temperature (30 min) [149]
Methoxymethyl (Mom)  **84**	$MgBr_2$ in Et_2O at room temperature (several hours) [150]
(Methylsulfanyl)methyl (Mtm)  **85**	Oxidation of sulfide into sulfonyl by H_2O_2 or ammonium molybdate or CH_3I followed by alkali hydrolysis [151]
Phthalimidomethyl  **86**	Zn/AcOH, HBr in AcOH, NaSPh, NH_2-NH_2 [152]
2-(Methylsulfanyl)ethyl[b),c)]  **87**	Oxidation of the sulfide moiety into sulfonyl group by H_2O_2/ammonium molybdate followed by alkali hydrolysis (12–24 h); alkylation to sulfonium salt by CH_3I followed by rapid hydrolysis at pH 10 (10-15 min) [153]
2-(4-Phenylazophenyl sulfonyl)ethyl  **88**	NaOH, pH 10–11, H_2O/dioxane [154]
2-Cyanoethyl (Cne)  **89**	10% aqueous K_2CO_3 [155]
2,2,2-Trichloroethyl (Tce)  **90**	Zinc in 90% AcOH (cleavage similar to Troc group) [156]

a) Catalytic hydrogenation reduces the oxo group to form the 2-phenylethyl ester that is stable to reductive cleavage.
b) Oxidation or alkylation generates an electron-withdrawing group β to the ester, treatment with base then cleaves the ester through fragmentation via β-elimination.
c) Acidolytic cleavage of protections can cause alkylation of the sulfur atom.

peptides can be cleaved without poisoning of the catalyst by carrying out catalytic hydrogenation in liquid NH_3 solvent at $-33\,^{\circ}C$ (boiling point of NH_3) for 16–18 h [163]. Benzyl esters are cleaved through CTH (e.g., Pd/C, 85% HCOOH) within minutes at 25 °C [47]. Anhydrous liquid HF treatment as well as by Birch reduction (Na-liquid NH_3) and saponification can also be employed to cleave the ester quantitatively [164]. Benzyl esters can be converted to TBDMS esters by treatment with Pd(II)acetate in the presence of TBDMS-Cl.

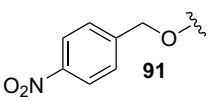
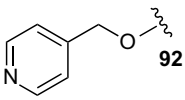
1.3.3
Substituted Benzyl Esters

Although benzyl esters offer excellent protection of the α-carboxy group, they are not completely stable to repetitive acidolytic steps used for deblocking N^{α}-Boc group (in Boc/benzyl protection strategy). To combat this limitation, substituted benzyl esters, which contain an electron-withdrawing group on the *para* position of the phenyl ring (e.g., 4-nitrobenzyl **91** and 4-pyridylmethyl **92**), have been introduced (Table 1.5). The substitution destabilizes the benzyl cation, which is a key intermediate during acidolytic cleavage, thus increasing the resistance of the ester to acids. However, the substitution increases the lability of the ester to alkaline hydrolysis.

1.3.4
tert-Butyl Ester

tert-Butyl esters are the widely used α-carboxy-protecting groups due to their remarkable stability to bases and nucleophiles as well as to catalytic hydrogenation, which make them suitable to be used in combination with Fmoc and Z groups [167]. They possess cleavage characteristics similar to Boc group. They are stable to weak acidic conditions that cleave extremely acid sensitive groups like Bpoc, Nps, and Trt. Stability of *tert*-butyl ester to nucleophiles when compared to *n*-alkyl esters can be attributed to

Table 1.5 Substituted benzyl esters.

Substituted benzyl ester	Preparation	Cleavage conditions
4-Nitrobenzyl (Nbz) [165]  **91**	Azeotropic distillation of amino acids with *p*-nitrobenzyl alcohol in toluene	Catalytic hydrogenation, reduction with Zn/AcOH, $SnCl_2$/DMF/AcOH, alkali hydrolysis
4-Pyridylmethyl (Pic) [166]  **92**	Treatment of N^{α}-protected amino acids with (4-chloromethyl) pyridine in the presence of TEA DCC-mediated esterification with pyridine-4-methanol	Catalytic hydrogenation, Na/liquid NH_3, 1 N NaOH in dioxane

the bulkiness of the group, which offers resistance to substitution at the ester carbonyl. However, exposure to high concentration of base for a long time can hydrolyze the ester.

tert-Butyl esters of amino acids as well as N^{α}-protected amino acids are prepared by treatment with isobutene in organic solvent in the presence of H_2SO_4 or HCl or TosOH [168]. Transesterification with commercially available *tert*-butyl acetate is also an efficient method of preparation [169]. Both these methods cause *O*- and *S*-alkylations. Hence, the hydoxy groups of N^{α}-Z-Thr/Ser are protected (with acetoacetyl group removable by treatment with hydrazine in EtOH) before esterification. Treatment of silver salts of N^{α}-protected amino acids with *tert*-butyl iodide is a mild method of preparation [170]. A combination of Boc-F and DMAP in CH_2Cl_2-*tert*-butanol (room temperature, 4 h) [171] and *tert*-butyl bromide in the presence of K_2CO_3 [172] also produce good yields of *tert*-butyl esters of Z-amino acids. Direct esterification of N^{α}-protected amino acids with *tert*-butanol can be done in the presence of $MgSO_4$ and H_2SO_4 [173] or $(Boc)_2O$ and catalytic amount of DMAP [174]. Alcoholysis of the mixed anhydride intermediate (generated by treatment of N^{α}-protected amino acid with 2,4,6-trichlorobenzoyl chloride in the presence of TEA) with *tert*-butanol also yields the esters [175]. Amino acid *tert*-butyl esters (distillable liquids) are stable and can be stored for months. Further, the free amino dipeptide esters do not undergo ring closure to DKP. Essentially, the reagents used for Boc cleavage can be used to cleave *tert*-butyl esters. Similar to the cleavage of Boc group, *tert*-butyl cation or *tert*-butyl trifluoroacetate formed during acidolysis can alkylate nucleophilic sites, which is suppressed by the addition of scavengers. Selective removal of the Boc group in the presence of *tert*-butyl ester can also be carried out (see Section 1.2.2.4.1).

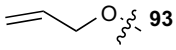
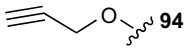
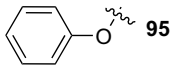
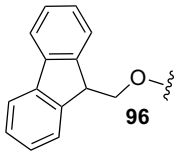
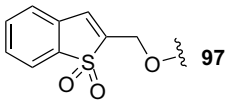
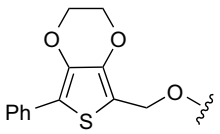
1.3.5
Other Acid-Labile Esters

Many acid-labile alkyl esters have been developed. In the order of increasing resistance to acidolysis, these esters can be arranged as: trityl (Trt) < diphenylmethyl (Dpm) = *tert*-butyl (*t*Bu) = 4-methoxybenzyl (Mob) = pentamethylbenzyl (Pmb) = trimethylbenzyl (Tmb) = phthalimidomethyl (Ptm) = 9-anthrylmethyl (Am) < benzyl [1]. 1-Adamantyl (1-Ada) esters have acid sensitivity similar to the Boc group, but are stable to treatment with 4 M HCl in anhydrous dioxane at room temperature for 25 min, a condition that removes the Boc group. Other important carboxy protections are summarized in Table 1.6.

1.3.6
Temporary α-Carboxy Protection

Temporary or transient α-carboxy protections are employed to suppress the activation of the carboxy group generally during the synthesis of short peptide acids or introduction of N^{α}-urethane protections. TMS esters are the commonly used temporary carboxy protections. They are quite stable in a nonaqueous medium but are instantaneously hydrolyzed upon exposure to very mild acid or base, or only to water. Hence, the ester can be quantitatively cleaved during aqueous work-up itself.

Table 1.6 Selected carboxy protections.

Protection	Cleavage conditions
Allyl  **93**	Similar to Aloc group Pd(0)-catalyzed allyl transfer in the presence of nucleophile [176]
Propargyl  **94**	Formation of alkyne–cobalt complex by reaction with complexes such as $Co_2(CO)_8$ followed by acidolysis with TFA [177]
Phenyl  **95**	Rapid saponification by treatment with alkali (these esters show weak acylating activity) [178]
9-Fluorenylmethyl (Fm)  **96**	Similar to Fmoc group[a)]
1,1,-Dioxobenzo[*b*]thiophene-2-yl (Bsm)  **97**	Similar to Bsmoc group[a)]
Phenyl-3,4-ethylenedioxythiophene (EDOT) 	0.01–0.5% TFA in DCM (the ester can be selectively removed over Boc) [179]
Amides α-carboxamides	Enzymatic hydrolysis through peptide amidase isolated from flavedo of oranges (optimum pH range: 6.8–8.4)
Tertiary amides	Potassium *tert*-butoxide (in water) in Et_2O selectively acts on tertiary amides without hydrolyzing peptide bonds (secondary amides) [180]

a) The combination of the Fm ester-Bsmoc group and Bsm ester-Fmoc group permits selective modification of the protections (β-elimination versus Michael acceptor units) by appropriate choice of the base; 2% TAEA and DBU remove only Bsmoc and Fmoc units, respectively.

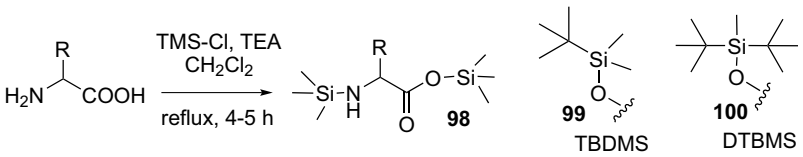


Figure 1.39 Silyl ester protection.

TMS esters of amino acids are obtained as *N,O*-bis-silyl derivatives **98** by the treatment of amino acids with TMS-Cl in the presence of TEA in refluxing DCM for 4–5 h (Figure 1.39) [181]. With Ser, Thr, and Tyr, the trisilylated derivative is formed. The *N,O*-bis-silyl amino acids are soluble in organic solvents, but decompose gradually upon standing. They are used as such for reactions without isolation. TBDMS **99** and di-*tert*-butylmethylsilyl (DTBMS) **100** esters (Figure 1.39) are comparatively more resistant to hydrolysis due to the presence of bulkier substitutions on the silicon atom [182, 183].

1.3.7
α-Carboxy Protectors as Precursors to Useful Amino Acid Derivatives: Formation of Acid Hydrazides

The esters of N^{α}-amino acids and peptides, particularly methyl and ethyl esters, can be directly converted into synthetically useful amino acid derivatives such as amino acid hydrazides **101**, which in turn can be converted to acid azides **102** or diimides (Figure 1.40) that are effective peptide coupling agents [184]. Diazotization of hydrazides with an N^{α}-Boc residue is also possible, although the reaction is carried out in the presence of HCl due to the very low temperature ($-30\,^{\circ}C$) employed.

1.4
Side-Chain Protection

1.4.1
ω-Amino Group of Diamino Acids

The ω-amino group of diamino acids, Lys and the nonproteinogenic Orn, 1,3-diaminobutyric acid (Dab), and 1,2-diaminopropionic acid (Dap), is strongly nucleophilic and entails compulsory protection irrespective of the method of peptide bond

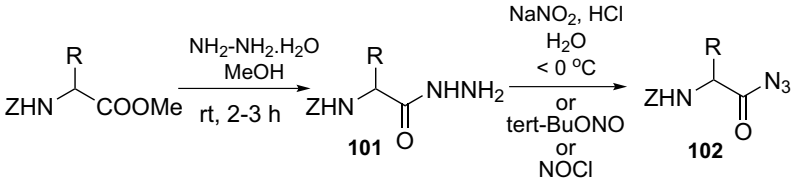


Figure 1.40 Preparation of Z-amino acid azides from methyl esters.

formation. In fact, the ω-amino group is more nucleophilic than the α-amino group due to the absence of a geminal electron-withdrawing group as in case of the latter. Fundamentally, all the groups employed as N^{α} protection can be used as N^{ω} protection. However, the stability and sometimes even the chemoselectivity of the N^{ω} and N^{α} protections differ from each other. Since the ω-amino group is a stronger nucleophile, the protection on it is more stable (to acids and bases), except in the case of the comparatively less nucleophilic β-amino group of Dap. Nonetheless, there is more freedom in the choice of a protecting group for the ω-amino group than that for the α-amino group. Since the vicinity of the ω-amino group is devoid of a sensitive chiral center or an activated carboxy moiety, the N^{ω} protection generally has a lower tendency to cause racemization or offer steric hindrance to coupling. Hence, acyl-type and bulkier groups, which are less useful for N^{α} protections, pose no difficulty as N^{ω} protectors.

Symmetrically protected diamino acids with identical N^{α},N^{ω} protection (e.g., Fmoc-Lys(Fmoc)-OH) can be synthesized in a straightforward manner by using 2 equiv. of an acylating agent. However, these compounds are not very useful for peptide synthesis since selective removal of N^{α} protection from them for subsequent acylation is not always successful (a rudimentary way to address this is to exploit the difference in the stabilities of N^{ω} and N^{α} protections, and deblock the latter with mild reagents with an acceptable level of selectivity). Therefore, only diamino acids that contain orthogonal N^{α},N^{ω} protections, and thus allow for selective removal of the N^{α} protection and in turn for chain extension in a linear fashion, are employed for peptide synthesis. The widely adapted approach to introduce differential N^{α},N^{ω} protections is the complexation method (Figure 1.41). Here, the α-amino group is made unreactive by trapping it in a Cu(II) complex and the free ω-amino group is acylated to introduce the N^{ω} protection. The complex is later decomposed by treatment with H_2S, Na_2S, thioacetamide or ethylenediaminetetraacetic acid (EDTA) or 8-quinolinol to liberate the α-amino group, which can then be acylated with the second reagent to introduce the N^{α} protection [185]. When acid stable N^{ω} protections are present, 6 N HCl can be used for decomposition of the copper complex. This method can also be executed with $BF_3{\cdot}Et_2O$ as the complexating agent. However, the approach is not successful in the case of Dap since it can form a six-membered

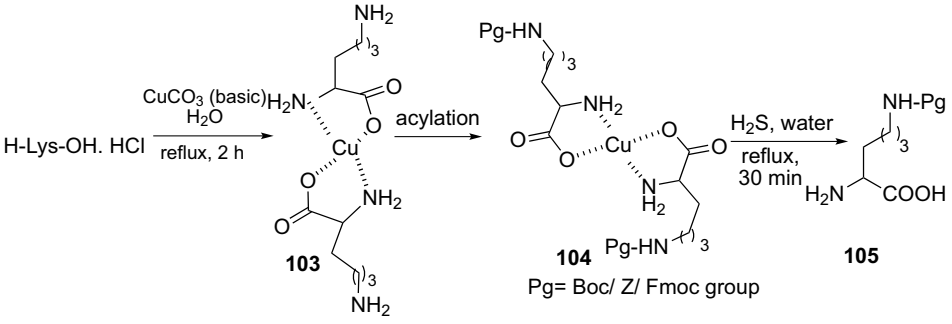


Figure 1.41 N^{ω}-Protection of Lys through complexation.

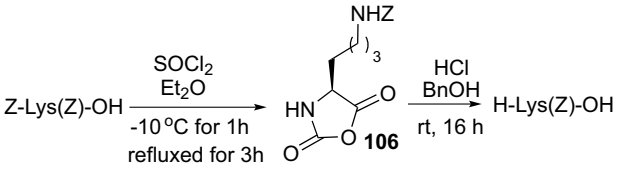


Figure 1.42 Preparation of H-Lys(Z)-OH from Z-Lys(Z)-OH.

chelation complex (in which the ω-amino group is trapped). Another approach is to make use of the greater nucleophilicity of the ω-amino group and selectively acylate it with moderately active reagents. This has been applied to introduce N^{ω}-For, Tfa, Boc, and Z groups by using *p*-nitrophenylformate (For-ONp), thioethyl trifluoroacetate, *tert*-butyloxycarbonyl-4-nitrobenzoate, and benzyloxycarbonyl phenolate, respectively [186]. In some cases, symmetrically substituted diamino acids such as Z-Lys(Z)-OH can be converted into the corresponding NCAs (Lys(Z)-NCA **106**) with the loss of only the N^{α} protection (Figure 1.42). The NCA can be hydrolyzed later to obtain ω-amino-protected diamino acids [187]. The widely used protections of the ω-amino group are summarized in Table 1.7.

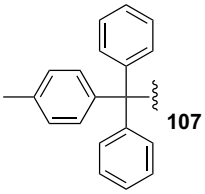
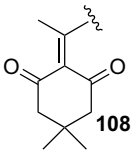
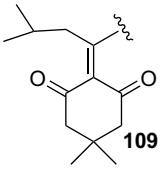
1.4.2 Guanidino Group of Arg

Ideally, all the three nitrogens (N^{δ}, N^{ω}, and $N^{\omega\prime}$) of the guanidino group in Arg have to be protected to completely suppress the nucleophilicity of the group. The unprotected δ, ω′, and ω-nitrogens can be involved in the formation of δ-lactams (**110**), cycloheptane derivatives (**111**), and deguanidilation of Arg to Orn (**112**), respectively (Figure 1.43). However, an ideal protection represented by N^{δ},N^{ω},$N^{\omega\prime}$-*per* derivatized guanidino group has not yet been devised (except Trt-Arg(Trt)$_3$-OMe, but its utility in peptide synthesis is yet to be established). Hence, in practice, N^{ω}- or $N^{\omega\prime}$-monoprotected and N^{δ},N^{ω}- or N^{ω},$N^{\omega\prime}$-bis-protected Arg derivatives are employed. The effectiveness of the protection is determined by the electron-withdrawing property or bulkiness (or by a combination of both these factors) of the protecting group(s), and on factors like synthetic strategy and activation method.

1.4.2.1 Protection Through Protonation

The strongly basic guanidino group ($pK_a = 12.5$) exists in its protonated form for most of the time in peptide coupling and hence is protected against side-reactions. Protonation is carried out with HCl, perchlorate pyridinium salt, and even with weakly acidic HOBt, but most effectively by HBr (due to the counter-ion effect) [196]. However, the N^{ω}-protonated Arg can be partially deprotonated during coupling by moderately basic components, even by H-Pro-*t*Bu, in the reaction mixture. In addition, the C-terminal residue is likely to cyclize to give a δ-lactam even in the protonated form.

Table 1.7 ω-Amino protections of diamino acids.

Protections	Stability	Cleavage
For		Methanolic HCl (room temperature) [188]
Tfa	Acids	Alkali, 1 M piperidine in water [189]
Mtt	Bases and nucleophiles	1% TFA in DCM [190]
 **107**		
Boc	Catalytic hydrogenation, bases and nucleophiles	Neat TFA or 25–50% TFA in DCM [191]
Z(2-Cl)	Bases, nucleophiles, mild acids	Catalytic hydrogenation, anhydrous liquid HF [192]
Dde[a)]	Acids, piperidine, DBU	2% Hydrazine hydrate in DCM [193]
 **108**		
ivDde (or Ddiv)[a)]	Acids, piperidine, DBU	2% Hydrazine hydrate in DCM [194]
 **109**		
Nbs	Acids, bases	β-Mercaptoethanol, DBU in DMF [195]

a) Migration of the Dde moiety on to the ω-amino group of neighboring unprotected diamino acid residues takes place during piperidine-mediated Fmoc removal. This migration is suppressed with the ivDde group.

1.4.2.2 Nitration

$Arg(NO_2)$ is commercially available and can also be prepared by the action of a nitrating mixture on Arg (Figure 1.44). The α-amino group of $Arg(NO_2)$ can be acylated in the usual manner to obtain N^{α}-protected $Arg(NO_2)$ [197]. The strong

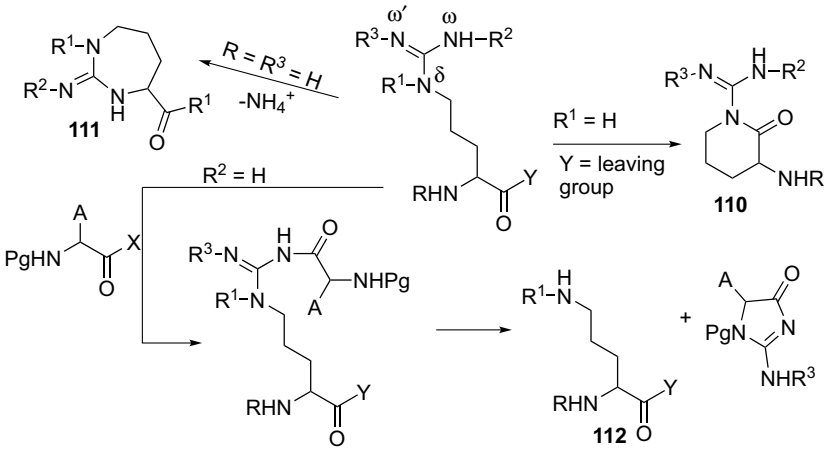


Figure 1.43 Side-reactions of unprotected Arg residues.

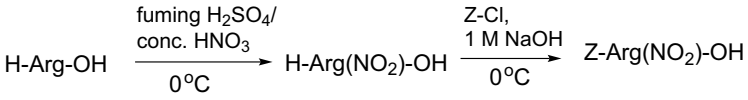


Figure 1.44 Preparation of Arg(NO_2) and N^{α}-protected Arg(NO_2).

electron-withdrawing property of the NO_2 group satisfactorily suppresses δ-lactam formation and N^{ω}-acylation, although a few exceptions have been found [197b,c]. The group is stable to mild acids and bases and is removed by catalytic hydrogenation, CTH (Trp can also undergo hydrogenation) and reduction by $SnCl_2$ [198]. On-resin removal of the group can be done via anhydrous liquid HF treatment. Other common Arg protectors are summarized in Table 1.8.

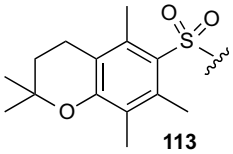
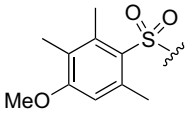
1.4.2.3 **Arg Precursors**

An Orn residue in peptides can be converted to an Arg residue through guanidilation of the δ-amino group by reagents such as N,N^{1}-bis(alkoxycarbonyl)thiourea **115**, N,N^{1}-bis(alkoxycarbonyl)-2-triflylguanidine **116**, or N,N^{1}-bis(alkoxycarbonyl)pyrazole-1-carboximidamide **117** (Figure 1.45) [206]. However, site-specific guanidilation in the presence of multiple diamino acid residues requires an additional level orthogonality in the protection of ω-amino groups. Also, guanidilation of multiple Orn residues is difficult.

1.4.3 **Imidazole Group of His**

Unprotected C-terminal His residues are racemized (e.g., His acid azide **118**) either by an autocatalyzed α-proton abstraction (by the π-nitrogen of the imidazole ring acting as a base) followed by enolization or by the formation of optically labile

Table 1.8 Arg protections (selected examples).

Protection	Cleavage conditions
N^{ω}-Ts[a]	HF/anisole at 0 °C, fluorosulfonic acid/anisole, Na/liquid NH_3) [199]
N^{ω}-2,2,5,7,8-Pentamethylchroman-6-sulfonyl (Pmc)[b]	TFA with several combinations of scavengers (i.e., anisole, PhSMe, Ph-OH, iPr$_3$SiH, and ethane-1,2-dithiol) [200]
 **113**	
N^{ω}-Pbf[b]	TFA/phenol in the presence of iPr$_3$SiH [19]
N^{ω}-4-Methoxy-2,3,6-trimethylbenzenesulfonyl (Mtr)	95% TFA/thioanisole; TFA/thioanisole in the presence of phenol and thiols [201]
 **114**	
N^{ω},$N^{\omega'}$-bis-Boc	TFA in the presence of scavengers [202]
N^{ω}-Boc	TFA in the presence of scavengers [203]
N^{δ},N^{ω}-bis-Adoc[c]	Neat TFA [204]
N^{δ},N^{ω}-bis-Z	Catalytic hydrogenation [205]

a) Deprotection of amino group of Arg(Ts) can lead to Orn formation, Na/liquid NH_3 treatment may cause desulfurization, racemization, and several decomposition reactions.
b) Pbf causes lower level of sulfonylation of Trp than Pmc.
c) α-amino free Arg(Adoc)$_2$ residues cyclize to cycloheptane derivatives in MeOH.

imidazolides **120** (Figure 1.46). In addition, the imidazole group of His undergoes acylation, in which case active acyl imidazoles that can cause undesired acyl transfers are formed.

In solution the His derivatives exist as a mixture of rapidly interconverting (through proton exchange) tautomers. Upon reaction with an electrophile, mono-

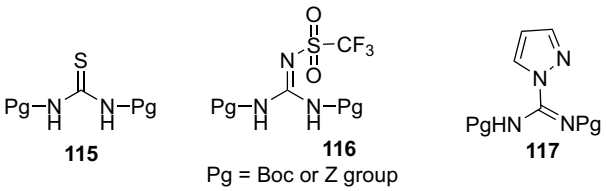


Figure 1.45 Guanidilating agents for conversion of Orn to Arg.

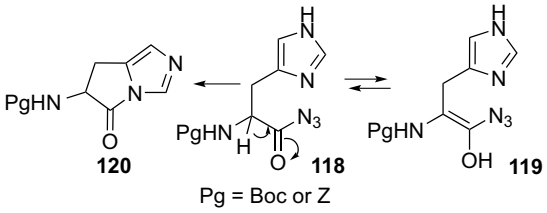


Figure 1.46 Racemization of unprotected His.

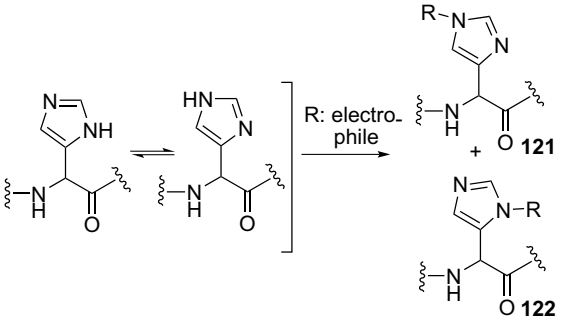


Figure 1.47 Electrophilic substitution of the indole ring of unprotected His.

substituted products are formed predominantly over N^{τ},N^{π}-disubstituted products. The product is typically a 3 : 1 mixture of stable N^{τ}-His **121** and N^{π}-His **122** (Figure 1.47). They are generally inseparable, with few exceptions such as the separation of the N^{τ} isomer (the major product) from the mixture of N^{α},N^{τ}-bis-Boc-His-OMe and N^{α},N^{π}-bis-Boc-His-OMe [207]. The proportion of the regioisomers formed can vary depending on the nature of the electrophilic reagent, structure of the substrate and reaction conditions, but in majority of the cases, the N^{τ} derivative predominates. Access to single N^{τ} isomers is advantageous as they can be used as substrates for the preparation of N^{π} derivatives that are usually not directly obtainable by electrophilic substitution of unprotected His. Preparation of N^{α}-Boc,N^{π}-Bom-His-OH **125** by employing N^{α},N^{τ}-bis-Boc-His-OMe **123** as starting material is shown in Figure 1.48 [207]. Formation of N^{τ},N^{π}-disubstituted products is usually rare, unless an excess amount of electrophilic reagent or unhindered reagents are used.

The presence of an electron-withdrawing group on the N^{π} position prevents autocatalyzed racemization. For instance, it was observed that the activated N^{α}-Z,N^{π}-

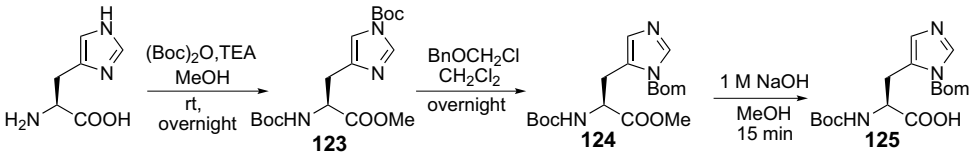
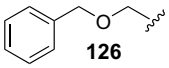


Figure 1.48 Preparation of N^{π}-Bom-substituted His.

Table 1.9 His protections.

Group	Stable to	Cleavage Conditions
N^{τ}-Trt	Bases and nucleophiles	Anhydrous liquid HF, HBr/AcOH [25, 209]
N^{π}-Benzyloxymethyl (Bom)	Bases and nucleophiles and TFA	Hydrogenolysis, acidolyis (addition of scavenger required to trap the byproduct HCHO formed during cleavage) [207]
 **126**		
N^{π}-Boc	Catalytic hydrogenation, bases and nucleophiles	TFA in the presence of scavengers [210]
N^{π}-Pac	Acids	Zinc in AcOH [208]

Pac-His-OH did not racemize, while the corresponding N^{τ} isomer did [208]. Nonetheless, even an N^{τ} substitution can be helpful in minimizing racemization since it decreases the nucleophilicity of the N^{π}-nitrogen by its electron-withdrawing effect. However, this is not always guaranteed as the level of suppression can still depend upon factors like the activation method. The widely used His-protecting groups are summarized in Table 1.9.

1.4.4 Indole Group of Trp

The unprotected indole group of Trp can undergo alkylation, sulfonylation, oxidation (to produce oxyindolyl derivative **127**), and dimerization (due to self-acylation by protonated Trp) during acidolytic cleavage of protections (Figure 1.49). *tert*-Butylation and nitrosation of the indolyl moiety is observed during Boc group removal and diazotization of acid hydrazides of Trp, respectively. Addition of scavengers during cleavage can significantly bring down the extent of these side-reactions. The

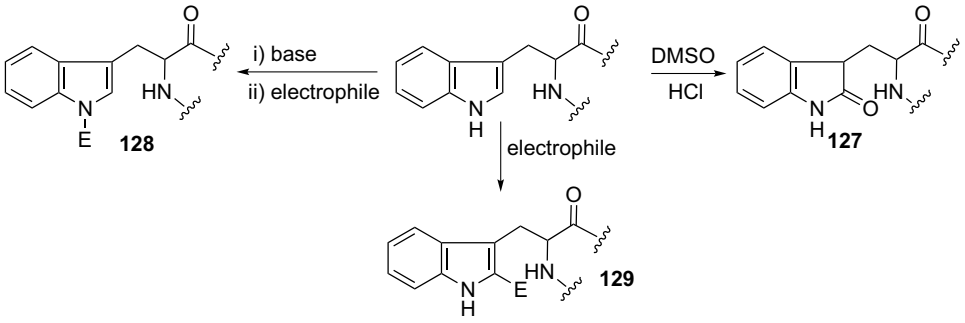


Figure 1.49 Side-reactions of unprotected Trp.

expulsion of air and oxidants and pretreatment of the solution of Trp with indole can avoid oxidation. Both 1- (in strongly basic medium **128**) and 2-positions **129** of the indole residue are susceptible to electrophilic substitution. However, in the proposed protection strategy, an electron-withdrawing group is placed on the ring nitrogen (1-position) – a method that also ensures that the electron availability in the heteroaromatic ring is decreased, thereby protecting the 2-position simultaneously.

The N^{in}-For group is stable to mild acids, and cleaved by treatment with hydrazine, piperidine in DMF, and anhydrous liquid HF-thiols [211]. The N^{in}-Boc group is stable to bases and catalytic hydrogenation, and is removed by treatment with TFA [212]. However, it is more stable to acidolysis and, further, the cleavage results in a stable carbamic acid intermediate, which undergoes decarboxylation slowly. The process can be accelerated by exposure to aqueous acids, which is usually at the work-up stage. In any case, the presence of an electron-withdrawing carboxy group on the *in*-nitrogen provides extended protection through the synthesis. The cyclohexyloxycarbonyl (Hoc) group is remarkably stable to bases and is removed by anhydrous liquid HF/*p*-cresol. The N^{in}-Aloc group is stable to acids and is removed by a Pd(0)-catalyzed allyl transfer reaction [213]. The group is labile to piperidine but stable to DBU. Hence, in the presence of this group, Fmoc is deblocked using DBU and not piperidine.

1.4.5
ω-Amido Group of Asn and Gln

The ω-amido group of C-terminal Asn and Gln (to lesser extent) is converted to a cyano group forming nitrile **130**, when the activation is carried out with carbodiimides or uronium-type reagents, or, to some extent, even with mixed anhydrides (Figure 1.50). However, the ω-amido group of N-terminal and as well as the *endo* Asn/Gln residues (as well as α-carboxamides) do not undergo dehydration. This suggests that dehydration occurs via a cyclic intermediate (an isoimide) formed by the intramolecular attack of the ω-amide oxygen on an activated carboxy group. Activation of Asn and Gln also leads to the formation of stable imides. The aspartimides **131** can be aminolyzed to Asn-containing peptides, but in the process β-aspartyl peptides are also formed. The imides can also be optically labile leading to racemization. The unprotected α-amino free Gln residues undergo cyclization to pyroglutamic acid **132** in acidic media (Figure 1.51). These side-reactions can be minimized by protecting the amido group of Asn and Gln. Also, the ω-amido protections increase the solubility of glutamine peptides, which are known to aggregate otherwise due to intermolecular

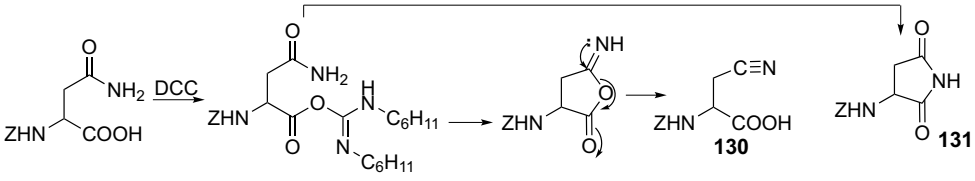


Figure 1.50 Formation of nitrile and aspartimide from unprotected Asn.

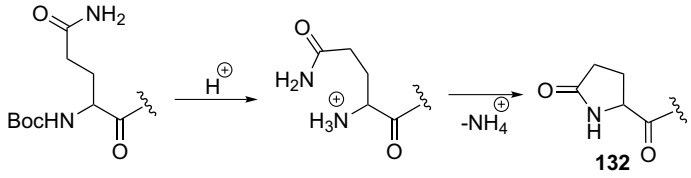


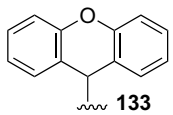
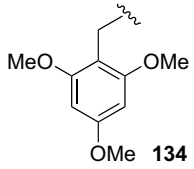
Figure 1.51 Acid-mediated formation of pGlu.

hydrogen bonding involving the unprotected ω-amido group. The widely used Asn and Gln protections are shown in Table 1.10.

1.4.6
β-Thiol Group of Cys

The thiol group of Cys is vulnerable to attack by electrophiles. Acylation of the thiol function of Cys during peptide coupling is not a serious concern since the *S*-acyl Cys (or any thioesters *per se*) are too reactive to nucleophiles and hence the transformation can be easily reversed. When *S*-acylation of N-terminal Cys takes place, the resulting thioester readily rearranges to a Cys peptide via an $S \rightarrow N$ acyl migration. In fact, this acyl transfer reaction has formed the basis for the development of native chemical ligation, a technique useful for linking two unprotected peptide fragments [217]. Self-

Table 1.10 ω-Amide protections (selected examples).

Protection	Stable to	Cleavage conditions
Trt	Catalytic hydrogenation, alkali, bases, and nucleophiles, dilute acids	95% TFA (10 min); when α--amino group is free, detritylation takes longer duration [214]
9H-Xanthen-9-yl (Xan)  **133**	Catalytic hydrogenation, bases	TFA/anisole (72 °C, 30 min), HBr/AcOH (room temperature, 1–2 h) [215]
2,4,6-Trimethoxy-benzyl (Tmb)  **134**	Catalytic hydrogenation, bases	TFA/anisole (room temperature, 5 min), HBr/AcOH (room temperature, 1–2 h), [216]

acylation of activated Cys can be prevented via a temporary and internal protection through conversion into thiolactones. The latter can be opened up by amines to yield a peptide incorporating Cys [218]. The unprotected thiol function can be irreversibly alkylated by electrophiles generated during the acidolytic removal of protections (Boc and Z groups). Peptides that contain N-terminal Cys can condense with HCHO released during the HF-mediated removal of the N^{π}-Bom group and form thiazolidine carboxylic acids (Thz peptides). The unprotected thiol moieties are also prone to oxidative formation of disulfide. In the case of the synthesis of peptides containing cystine (a dimer of Cys cross-linked by a -S–S- linkage), the regioselective formation of the disulfide bond is an essential step. Nevertheless, unprotected Cys(s) do not always guarantee regioselective disulfide formation. Consequently, the thiol group of Cys is compulsorily protected during synthesis. The important thiol protections are summarized in Table 1.11.

1.4.6.1 **Common Side-Reactions with S-Protected Cys Derivatives**

1.4.6.1.1 **Racemization** The carboxy-activated *S*-protected Cys and Cys residues anchored to resin by an ester linkage **139** (and not Cys α-carboxamides and *endo* residues) are racemized even during rapid peptide couplings due to high rates of base-catalyzed enolization. This can be minimized by base-free couplings and by the selection of suitable *S*-protectors. The rate of epimerization during SPPS using N^{α}-Fmoc protection has been found to vary with different thiol protections. The minimum rate was found with *S-tert*-butyl protection and the maximum with the *S–S-tert*-butyl group.

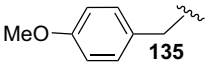
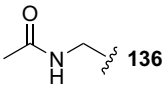
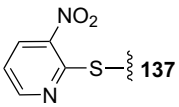
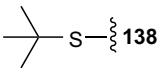
1.4.6.1.2 **β-Elimination** Protected Cys are converted to ΔAla residues **140** by the action of strong bases or strong acids through a β-elimination process involving the α-proton and the protected thiol function. The resultant ΔAla residues are reactive to nucleophiles and can form adducts such as β-(1-piperidyl)Ala **141** (Figure 1.54).

1.4.6.1.3 **Oxidation** Sulfide, disulfide as well as thioacetal-based protections are oxidized to the corresponding sulfoxides in the presence of peroxides. The process is also feasible by air oxidation.

1.4.6.2 **Synthesis of Peptides Using Cystine as "Self-Protected" Cys**

Symmetrical cystine derivatives can be employed for the synthesis of peptide dimers cross-linked by a disulfide bond and peptides containing a single Cys residue. In the case of the latter, the disulfide bond is reduced (using β-mercaptoethanol) to liberate two molecules of the Cys peptide. An application of this method can be seen in the synthesis of "H peptide" [227]. The method can also be extended to make asymmetric Cys peptides and also cyclic cystine "loops" by using unsymmetrical disulfides of Cys. The method is less successful for SPPS since acylation of resin bound amines with activated *N,N′*-bis-protected cystine which is often incomplete leading to the formation of mono-cystine peptides, which upon subsequent acylation form asymmetric Cys peptides. The major limitation of the method is the high degree of sensitivity of

Table 1.11 Selected β-thiol protections of Cys.

Protection Alkyl Type	Stable to	Cleavage[a] conditions
4-Methoxybenzyl (Mob)  **135**	TFA, bases,	$Hg(OAc)_2$ in TFA, anhydrous liquid HF, TFA/scavengers, I_2 [219]
Xanthyl	Bases and nucleophiles	Hg(II), anhydrous liquid HF (20 °C), $Ph_2SO/MeSiCl_3$ [220]
Trt	Bases and nucleophiles	TFA/H_2O or $DCM/isoPr_3SiH$, $AgNO_3$, I_2 [221]
Acetamidomethyl (Acm, Figure 1.52)  **136**	Bases, TFA, liquid HF (0 °C)	I_2, heavy metal ions, R-SCl, $Ph_2SO/MeSiCl_3$ [222]
3-Nitro-2-pyridylsulfanyl (NPys)  **137**	Anhydrous liquid HF	Thiols including free Cys [223]
tert-Butylsulfanyl[b]  **138**	TFA, bases, RSCl	Thiols, TFE [224]

a) Cys protections can be removed with concomitant formation of a disulfide linkage via I_2-mediated oxidation. This procedure (Figure 1.53), first developed for the cleavage of Acm group [225], has been successfully extended to other protections as well and further to selective removal of thiol protections (e.g., Trt) in the presence of the Acm group. Thallium(III) acetate can also be used for the removal of the Acm group with disulfide formation. An illustrative application of this procedure is the total synthesis of human insulin by the stepwise disulfide formation approach [226].

b) When the Acm group is present, thiols and thioethers are avoided as scavengers as they can cause disulfide exchange.

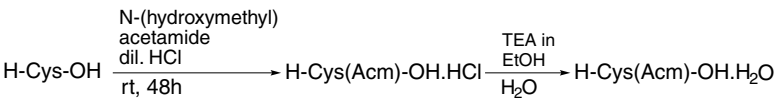


Figure 1.52 Preparation of *S*-Acm protected Cys.

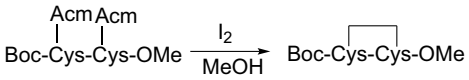


Figure 1.53 Oxidative removal of *S*-Acm protection of Cys with concomitant disulfide formation.

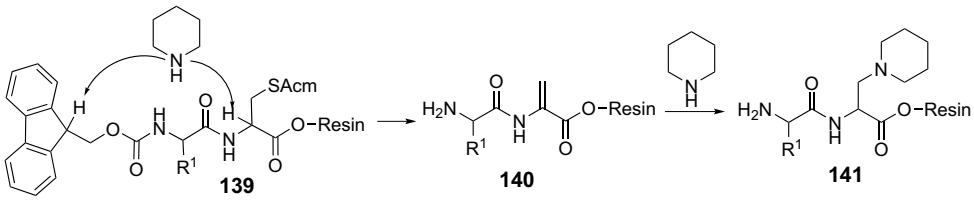


Figure 1.54 Racemization and β-elimination in *S*-protected Cys residues.

disulfide bonds to regular synthetic conditions. The -S–S- linkages are sensitive to oxidation and reduction. Disulfide exchange can also take place with scrambling of the -S–S- linkages in the case of asymmetric cystine peptides. This reaction is catalyzed by acids or bases or by a small amount of free thiols. Hence, exposure to conditions, which promote these side-reactions, should be avoided when working with cystine derivatives.

1.4.7
Thioether Group of Met

The methyl thioether moiety of Met is susceptible to oxidation and alkylation. The *S*-alkylated function can be internally displaced by the carboxy group to form a homoserine lactone. The favored method of protection of Met is oxidation of the thioether group to sulfoxide **142**. Oxidation of Met is associated with the formation of sulfone byproducts and a mixture of (2*S*,*R*)- and (2*S*,*S*)-sulfoxides due to asymmetric induction. Several reagents have been proposed for stereospecific and sulfone-free oxidation. Sodium perborate is efficient in terms of preventing sulfone formation but gives a mixture of epimers (Figure 1.55). The required *S*-Met(O) is isolated as the picrate salt by recrystallization. Met can also be oxidized in excellent yields by H_2O_2-mediated oxidation, but the reaction gives rise to varying amounts of epimeric sulfoxides [228]. The thioether moiety is regenerated by reduction with mercaptoacetic

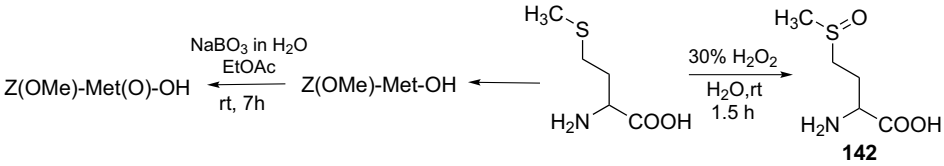


Figure 1.55 Protection of Met through oxidation.

acid [229]. TMS-SPh, *N*-methyl sulfanylacetamide, selenophenols, TMS-OTf, and NH_4I/TFA are the other reagents proposed for the reduction of the sulfoxide of Met.

1.4.8
Hydroxy Group of Ser, Thr, and the Phenolic Group of Tyr

The β-hydroxy group of Ser and Thr (to lesser extent) and the phenolic function of Tyr are acylated in the presence of excess of acylating agents, which is typically the case in SPPS and during difficult couplings. N-Terminal Ser can undergo double acylation by carboxy-activated aspartic acid ω-esters to give an eight-membered macrocycle containing amide and ester linkages. *O*-Acylation of Ser has been found to be promoted by the catalytic activity of the imidazole moiety of unprotected His. In addition, the hydroxy function of Ser/Thr undergoes acid-catalyzed dehydration to ΔAla derivatives. An intramolecular $N \rightarrow O$ acyl shift can take place in the presence of strong acids such as liquid HF and TfOH. However, this isomerization can be easily reversed by brief exposure to mild alkali (the principle has formed the basis for a peptide ligation route called the "click peptide" method). Problems associated with the usage of unprotected Ser as an acylating agent include self-acylation (which is more pronounced under strong activation conditions and long hours of standing) and formation of oxazolidin-2-ones. The problem of *O*-acylation is more serious in the case of Tyr due to the formation of the strongly nucleophilic phenoxide ion, which is also accessible to acylating agents without steric constraints. In addition, the phenolic function activates the aromatic ring to electrophilic substitution at the 3-benzyl position. Hence, scavengers are invariably used during acidolytic removal of protecting groups. The common protections of Ser and Thr are summarized in Table 1.12, and those of Tyr in Table 1.13.

Table 1.12 Protectors of the hydroxy group of Ser/Thr.

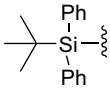
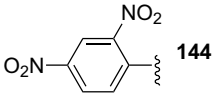
Protection	Stable to	Cleavage conditions
Alkyl type		
Benzyl	Acids, bases	Catalytic hydrogenation, liquid HF, TfOH [230]
tert-Butyl	Catalytic hydrogenation, bases	90% TFA in DCM, neat TFA [231]
Trt	Catalytic hydrogenation, bases	1% TFA in DCM [232]
Silyl ethers		
TBDMS	Bases, catalytic hydrogenation	TFA, TBAF [233]
TBDPS	Bases, catalytic hydrogenation, acids	TBAF, alkali [234]
 **143**		

Table 1.13 Protectors of the phenolic function of Tyr.

Group	Cleavage conditions
Alkyl ether	
Benzyl, Bzl	Catalytic hydrogenation, anhydrous liquid HF, TfOH, [235]
tert-Butyl, Trt	Mild acids
Aryl Ether	
2,4-Dinitrophenyl (Dnp)	Thiolysis, piperidine, DBU [236]
 **144**	
Silyl ether	
TBDMS	TBAF, mild acids [233]
Acyl esters	
Z(2-Br)	Super acid, piperidine [237]
Propargyloxycarboxyl	Benzyltriethylammonium tetrathiomolybdate in acetonitrile [238]

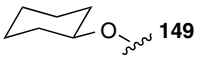
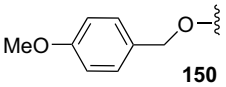
1.4.9 ω-Carboxy Group of Asp and Glu

Protection of the ω-carboxy group of Asp and Glu is required to avoid its activation during coupling (which would lead to branching of the peptide chain at Asp and Glu residues), and to minimize the formation of imides as well as pGlu derivatives. There are different synthetic routes to access ω-esters of Asp/Glu. These esters can be prepared in good yields by acid-mediated esterification of Asp and Glu [239]. Protonation of the α-amino group in acidic media has a deactivating effect on the adjacent α-carboxy group because the presence of a positive charge on the amino group prevents protonation (the key step in acid-catalyzed esterification) of the latter. Treatment of Asp and Glu with excess isobutene and catalytic TsOH in dioxane yields the monoesters (55–60% yield), with a ω/α ester ratio of 65 : 35 for Glu and 60 : 40 for Asp. The monoesters can be derivatized with N^{α}-Fmoc group and ω-*tert*-butyl esters of Fmoc-Asp/Glu can be obtained by crystallization from CH_2Cl_2/petroleum ether [240]. ω-Esters can also be synthesized by using Cu^{2+} complex of Asp and Glu or oxazolidin-5-ones of N^{α}-protected Asp/Glu in which the α-carboxy group is trapped through bonding [241, 242]. Hydrolysis of diesters of Asp and Glu in the presence of Cu^{2+} salts gives access directly to the ω-esters [243]. The most widely used ω-carboxy protections are listed in Table 1.14.

1.4.9.1 Aspartimide Formation

A persistent side-reaction during peptide synthesis is the base or acid-catalyzed cyclization of the ω-protected Asp residues into aspartimide. The homologous glutarimide is formed to a lesser extent. The extent of aspartimide formation is

Table 1.14 ω-Esters of Asp and Glu (selected examples).

ω-Esters	Cleavage
Benzyl	Catalytic hydrogenation, anhydrous liquid HF, saponification [244]
4-Nitrobenzyl	More stable to acidolytic cleavage than benzyl ester; cleaved by catalytic hydrogenation; TBAF; Na_2S [245]
Cyclohexyl (cHx)	Anhydrous liquid HF or MsOH [246]
 **149** *tert*-Butyl	
tert-Butyl	TFA in DCM, HCl in dioxane [247]
4-Methoxybenzyl	1% TFA in DCM [248]
 **150**	
Fm	20% Piperidine in DMF or 15% DEA in DMF [249]
Allyl	Similar to Aloc group [250]

high in syntheses employing Fmoc for N^{α} protection due to the high frequency of exposure of peptides to bases. Aspartimides are reactive to nucleophiles, and undergo hydrolysis to a mixture of α- and β-peptides **145** and **146**. When the nucleophile is piperidine, the corresponding α- and β-piperidine amides **147** and **148** are formed (Figure 1.56). An ideal ω protection is expected to prevent or at least minimize aspartimide formation. Generally, the ω-esters of primary alcohols are more prone to

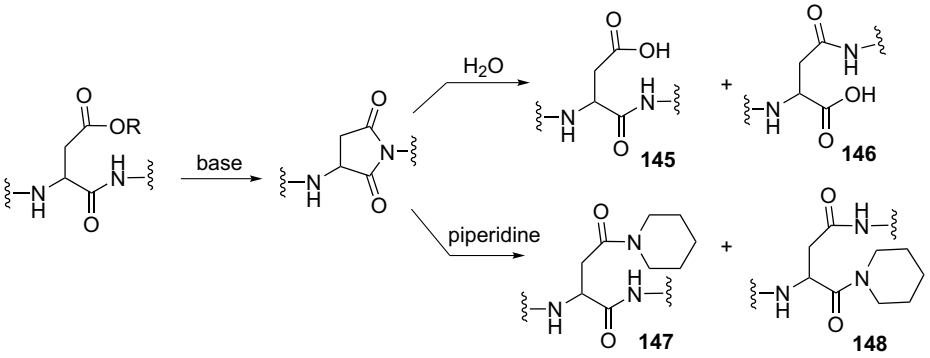
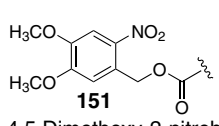


Figure 1.56 Formation of aspartimide and β-peptide and amide.

cyclization than that of secondary and tertiary alcohols. Hence, methyl and ethyl esters, and to some extent benzyl esters, are not suitable to prevent aspartimide formation. However, sterically hindered esters such as cycloalkyl esters (cyclopentyl, hexyl, heptyl, octyl, and dodecyl esters), menthyl esters, adamantyl esters, *tert*-butyl esters, Fm esters as well as allyl esters are resistant to aspartimide formation. Consequently, the classical alkyl esters are increasingly replaced by the more efficient cyclohexyl esters.

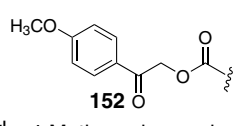
1.5
Photocleavable Protections

Groups that are fragmented by absorption of electromagnetic radiation have been developed as protective functions for peptide synthesis. The major advantages of using photocleavable protectors are their higher degree of orthogonality with other protections when compared to the conventional acid- or base-labile protecting groups, and the prospect of carrying out the deprotection reaction in neutral conditions, which is ideal to minimize a number of side-reactions. Photocleavable carboxamides **154** and **155** are attractive units as carboxy protections since they can be cleaved with excellent levels of selectivity [251]. The suitability of a photocleavable group is determined by the quantum efficiency and the degree of selectivity of the cleavage reaction, and the nature of the photoproducts formed during cleavage, which is expected to be inert. Typically, photocleavable groups are designed to be cleaved with irradiation with ultraviolet radiation with wavelength around 320 nm. Radiations of higher energy cause multiple fissions leading to a decrease in the selectivity level and those with lower energy (e.g., visible light) lead to photodegradation of the protecting group. Important photocleavable groups employed as α-amino protectors are shown in Figure 1.57. Photocleavable carboxy protectors are the esters derived from the alcohol component of the alkoxycarbonyl derivatives in Figure 1.57 [252–254].



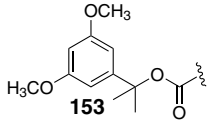
151

4,5,Dimethoxy-2-nitrobenzyl-oxycarbonyl (Nvoc)



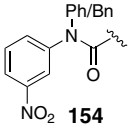
152

4-Methoxyphenacyloxy-carboxyl (Phenoc)

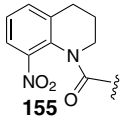


153

2-(3,5-Dimethoxyphenyl)prop-2-yloxycarbonyl (Ddz)



154



155

Figure 1.57

1.6 Conclusions

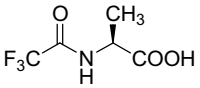
Since Emil Fischer's disclosure of the significance of the N^{α}-protecting group for the successful chemical assembly of a dipeptide, the protection of the functional groups of α-amino acids has been the topic of intense research. The breakthroughs achieved in the development of a new protecting group or the discovery of a new cleavage reagent/condition have eventually resulted in stretching the limits to new dimensions of the achievable size, level of purity, and quantity of synthetic peptides through solution-phase synthesis as well as SPPS. Investigations continue to be undertaken in the direction of describing new protecting groups and improved analogs, and efficient deprotection conditions. The important aspects are the development of new methods/reagents for the introduction of protections, increasing the homogeneity of protected amino acids, addressing solubility issues, and minimization of side-reactions during peptide coupling and cleavage steps. Several nonconventional methods such as microwave and ultrasonication-mediated synthesis, photochemistry and enzyme catalysis are being tried as new routes for introduction as well as removal of protectors. Progress is also being made in the standardization of methods with respect to meeting the principles of "green" synthesis through innovations such as development of water-soluble protectors, aqueous-phase reactions, and atom-economical synthetic methods. Protectors are also being optimized for their suitability in rapid peptide synthesis via microwave-SPPS.

A vast diversity of N^{α}, carboxy, and side-chain protections and cleavage reagents are currently available. It is not possible to identify an ideal protecting group or a combination. The choice of an appropriate protection/protection scheme depends on factors such as the sequence of interest, mode of synthesis (solution-phase or SPPS), protection strategy (global versus minimum protection), desired purity levels of peptides, scale, time, and cost. Nonetheless, since their inception, the urethanes have dominated as N^{α}-protecting groups for peptide synthesis and the trend appears to not change in the near future. The Fmoc/*tert*-butyl protection strategy is currently the most widely employed protocol for the synthesis of peptides through SPPS. However, the non-urethane-type protections retain importance in many cases. An example is the coupling of extremely sterically crowded amino acids, in which case the hindrance offered by the urethane appears to be the limiting factor for peptide coupling [19b]. Further, with the advent and popularization of peptidomimetics, the groups, which have not found appreciable level of utility as protective functions for conventional peptide synthesis, are being explored due to the vast diversity in reactions being carried out using amino acids as substrates. Also, there are indications that the protecting groups may not necessarily be viewed as inevitable extra appendages in substrates, but as units with synthetic value. Postsynthetic modifications wherein the protective function, instead of being removed, is directly converted to functional groups is being carried out [255].

1.7
Experimental Procedures

1.7.1
Protection Reactions

1.7.1.1 General Procedure for the Preparation of Tfa-Arg-OH [4]

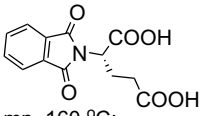


mp. 70 °C;
$[\alpha]^{16}_D$ = -60.7 (*c* 1, H_2O)

TEA (3.1 ml, 22.0 mmol) was added to a solution of Ala (2.0 g, 22.0 mmol) in MeOH (11 ml). After 5 min, ethyl trifluoroacetate (3.3 ml, 28.0 mmol) was added and the reaction was stirred for 24 h. The solvent was evaporated and the residue was dissolved in H_2O (35 ml) and acidified with concentrated HCl (approximately 4 ml). After stirring for 15 min, the mixture was extracted with EtOAC (30 ml × 4) and the combined organic layers were washed with brine (25 ml), dried ($MgSO_4$), filtered, and concentrated to leave a clear oil that upon subjecting to high vacuum for 24 h solidified into a hygroscopic solid. Yield: 87%.

Note: N^{α}-Tfa-amino acids, except Tfa-Ser and Tfa-Thr, are prepared efficiently by treating amino acids with TFAA in TFA solvent at –10 °C [4].

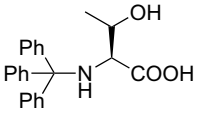
1.7.1.2 General Procedure for the Preparation of N^{α}-Phthaloyl Amino Acids using *N*-(Ethoxycarbonyl)phthalimide [256]



mp. 160 °C;
$[\alpha]^{25}_D$ = -48 (*c* 1, dioxane)

Finely powdered *N*-(ethoxycarbonyl)phthalimide (5.0 g, 22.8 mmol) was added to a suspension (0 °C) of Glu (2.4 g, 16.3 mmol) and Na_2CO_3 (4.2 g, 40.0 mmol) in H_2O (20 ml), and stirred at 0 °C for 5 min. The mixture was acidified to pH 2.5 with 6 M HCl and the resulting oil crystallized upon cooling. The product was filtered, washed with cold H_2O, and dried. Yield: 80%.

1.7.1.3 General Procedure for the Preparation of N^{α}-Trt-Amino Acids [25]



mp. 165 °C;
$[\alpha]^{25}_D$ = -6.7 (*c* 2, MeOH)

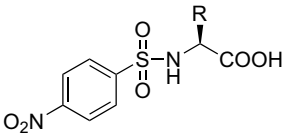
i) Preparation of TMS-amino acid: TMS-C1 (17.75 ml, 140.0 mmol) was added to a suspension of Thr (4.76 g, 40.0 mmol) in DCM (70 ml) and the mixture was refluxed for 20 min. Et_3N (19.51 ml, 140.0 mmol) in DCM (40 ml) was added after cooling to room temperature and refluxed again for 45 min. The TMS-amino acid was used directly for the next step.

ii) The mixture, obtained from the above step, containing TMS-amino acid, was cooled to 0 °C, anhydrous MeOH (2.43 ml, 60.0 mmol) in DCM (10 ml) was added dropwise, and the mixture was allowed to attain room temperature. Et_3N (5.58 ml, 40.0 mmol) was added followed by Trt-Cl (11.25 g, 40.0 mmol) in two portions over a 15 min period. The mixture was stirred for 5 h and then excess MeOH was added. The residue obtained after evaporation of the solvent was partitioned between EtOAc (50 ml) and a precooled 5% citric acid solution (50 ml). The organic layer was extracted with 1 N NaOH (20 ml × 2) and H_2O (20 ml × 2). The combined aqueous layers were washed with EtOAc (20 ml), cooled to 0 °C, and neutralized with glacial AcOH. The precipitated product was extracted with EtOAc (30 ml × 2), and the combined organic layers were washed with H_2O, dried over $MgSO_4$, and concentrated. The resulting residue was dissolved in EtOAc (20 ml) followed by the addition of Et_2NH (1 ml, 10.0 mmol) to obtain the diethylammonium salt. Yield: 67%.

Note:

- In the case of amino acids other than Ser, Thr, and Tyr, the corresponding *N,O*-bis-TMS amino acids can be analogously prepared using 2 equiv. of TMS-Cl.
- Trt-amino acids can also be prepared using Me_2SiCl_2 and Ph_2SiCl_2 in place of Me_3SiCl [25].

1.7.1.4 General Procedure for the Preparation of N^{α}-Ns-Amino Acids [257]



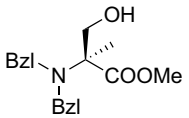
p-Nitrobenzenesulfonyl chloride (1.5 g, 1.6 mmol) was added slowly to a solution of the amino acid (1.0 mmol) in 1 N NaOH (0 °C) and the mixture, maintained at pH 9, was stirred for 2–3 h (TLC: $CHCl_3$/MeOH 90 : 10, v/v). The mixture was extracted with EtOAc (10 ml × 3). The aqueous phase was acidified to pH 2.0 with 1 N HCl and extracted with EtOAc. The organic layer was washed with H_2O and brine, dried over Na_2SO_4, and evaporated to afford the product.

Note:

- The side-chain-protected amino acids (i.e., Ns-Lys(Boc)-OH, Ns-Asp(O*t*Bu)-OH, Ns-Cys(Trt)-OH, and Ns-Asn(Trt)-OH) have been prepared using *p*-nitrobenzenesulfonyl chloride in presence of TEA in dioxane-H_2O [21].
- N^{α}-Nbs-amino acids are prepared through the treatment of the amino acid with 1.5 equiv. of Nbs-Cl in a mixture of 1 : 1 dioxane and 1 M NaOH. This is a modified

procedure originally employed for the preparation of benzylsulfonyl amino acids [258].

Bzl$_2$-α-Me-Ser-OMe [259]

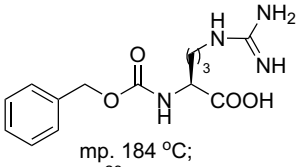


$[\alpha]^{23}_D$ = -34.3 (*c* 1.17, $CHCl_3$)

H-α-Me-Ser-OMe·HCl (2.96 g, 17.5 mmol) was dissolved in THF/DMSO (80 ml/20 ml), and $NaHCO_3$ (8.82 g, 105.0 mmol) and BzlBr (8.36 ml, 70.3 mmol) were added. The solution was refluxed overnight and H_2O (50 ml) was added, and the mixture was extracted with EtOAc (100 ml). The organic layer was dried over $MgSO_4$ and evaporated. The resulting residue was purified via flash chromatography (hexane/EtOAc (9 : 1) to hexane/EtOAc (6 : 4)). Yield: 64%. IR: 3455, 2952, 1728, 1454 cm^{-1}.

Note: The methyl ester can be hydrolyzed following the procedure described for closely related substrates by refluxing with LiI and NaCN in pyridine under N_2 atmosphere [259].

1.7.1.5 General Procedure for the Preparation of N^{α}-Z-Amino Acids



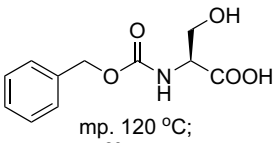
mp. 184 °C;
$[\alpha]^{23}_D$ = -9.3 (*c* 2, 1N HCl)

1.7.1.5.1 **Method A: Using Z-Cl [41a]** To a solution of Arg·HCl (21.3 g, 100 mmol) in 1 N NaOH (100 ml) maintained at 0 °C, Z-Cl (22.1 g, 130.0 mmol) and 2 N NaOH (55 ml) were added in portions, alternatively. The pH of the mixture was kept between 9.0 and 10.0. Stirring was continued for a further 2 h during which the pH drops to 7.0–7.5. The resulting precipitate was filtered, washed with cold H_2O (50 ml), and recrystallized from boiling H_2O (about 130 ml). Crystallization was completed in the cold (ice-H_2O bath). The product was air-dried, and then the powder was suspended in acetone (50 ml) and filtered, and washed with acetone (20 ml) and ether (50 ml). The purified material was dried *in vacuo* at 50 °C. Yield: 89.5%.

Note: Z-Cl is prepared by treating benzyl alcohol with phosgene (gaseous or in toluene solution (commercially available)) at −20 °C and continuing stirring for several hours and allowing the mixture to attain room temperature. The product is isolated after work-up [260].

Caution: Phosgene is a highly toxic gas and should only be used following strict safety measures.

1.7.1.5.2 Method B: Using Z-OSu [42]



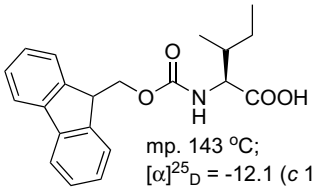
mp. 120 °C;
$[\alpha]^{26}_D$ = +5.7 (*c* 6, AcOH)

Z-OSu (1.24 g, 5.0 mmol) was added to a solution of Ser (525 mg, 5.0 mmol) and $NaHCO_3$ (420 mg, 5.0 mmol) in a mixture of H_2O (7 ml) and acetone (7 ml), and the whole was stirred overnight. The acetone was then evaporated and the solution was washed with DCM (3 ml × 2). The aqueous layer was acidified to pH 2.5 with concentrated HCl and extracted with EtOAc (5 ml × 3). The combined organic layers were washed with H_2O, dried, concentrated, and the residual product was recrystallized. Yield: 78%.

Note: Z-OSu is prepared by treating the DCHA salt of *N*-hydroxysuccinimide with Z-Cl for 12 h at room temperature [42].

1.7.1.6 General Procedures for the Preparation of N^{α}-Fmoc-Amino Acids

1.7.1.6.1 Method A: Using Fmoc-OSu [117]

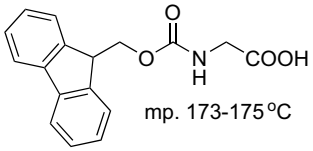


mp. 143 °C;
$[\alpha]^{25}_D$ = -12.1 (*c* 1, DMF)

Fmoc-OSu (165.0 g, 0.49 mol) was added to a solution of Ile (66.0 g, 0.50 mol) and Na_2CO_3 (53.0 g, 0.50 mol) in H_2O/acetone (1:1, 1.3 l) over a period of 1 h while the pH was kept at 9.0–10.0 by the addition of 1 M Na_2CO_3 solution. Stirring was continued overnight, EtOAc (2 l) was added and the mixture was acidified with 6 M HCl. The organic layer was washed with H_2O (1.5 l in four portions), dried, and concentrated to approximately 500 ml. On addition of petroleum ether, the product crystallized. Yield: 93%.

Note: Preparation of Fmoc-OSu: To a chilled solution of Fmoc-Cl (102.8 g, 0.40 mol) and *N*-hydroxysuccinimide in dry dioxane (700 ml) was added TEA (55.75 ml, 0.40 mol), and the mixture was stirred for 4 h. The TEA·HCl was filtered, washed with dioxane, and the combined filtrates were concentrated. The product was crystallized by the addition of petroleum ether. Melting point 148–149 °C [117].

1.7.1.6.2 Method B: Using Fmoc-Cl and *N*,*O*-bis-TMS-Amino Acids [119]

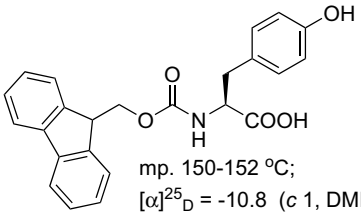


mp. 173-175 °C

TMS-Cl (9.52 ml, 75.0 mmol) was added to finely ground Gly (2.81 g, 37.5 mmol) suspended in DCM (87.5 ml) and the mixture was refluxed for 1 h and then cooled in an ice bath. Then DIPEA (11.3 ml, 65.0 mmol) and Fmoc-Cl (6.47 g, 25.0 mmol) were added. The solution was stirred with cooling for 20 min and warmed to room temperature for 1.5 h. The mixture was concentrated and partitioned between Et_2O (200 ml) and 2.5% $NaHCO_3$ (250 ml). The aqueous layer was washed with Et_2O (50 ml × 2) and the Et_2O layers were back-extracted with H_2O (25 ml × 2). The combined aqueous layers were acidified to pH 2.0 with 1 N HCl and extracted with EtOAc (75 ml × 3). The combined EtOAc layers were dried and concentrated to obtain the product that was recrystallized using EtOH/H_2O. Yield: 88%.

Note: For the preparation of Fmoc-Cl, 9-fluorenylmethanol (12.8 g) was added slowly to a chilled solution of phosgene (7.12 g) in DCM (75 ml), and the mixture was stirred for 1 h in an ice bath and then allowed to stand for 4 h at ice-bath temperature. The solvent and excess phosgene were removed *in vacuo* to leave an oil which crystallized after several hours. The resulting product was recrystallized twice from ether. Yield: 86%; melting point 61.5–63 °C [261].

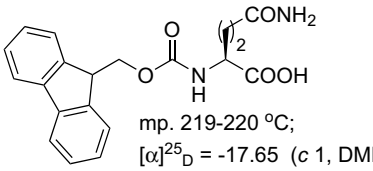
1.7.1.6.3 Method C: Using Fmoc-Cl in the Presence of Zinc Dust [118]



mp. 150-152 °C;
$[\alpha]^{25}_D$ = -10.8 (*c* 1, DMF)

Tyr (1.81 g, 10.0 mmol) was dissolved in a minimum quantity of 1 N HCl in MeCN followed by the addition of activated zinc dust (prewashed with dil. HCl and dried) in small portions until the reaction mixture attained neutral pH. A solution of Fmoc-Cl (2.7 g, 10.0 mmol) in MeCN (10 ml) and zinc powder (650 mg, 10.0 mmol) were added and stirred at room temperature for about 20 min. The mixture was filtered, washed with MeCN and the filtrate was evaporated. The residue was dissolved in 5% Na_2CO_3 (20 ml) and washed with ether (10 ml × 3). The aqueous layer was acidified to pH 2.0 with 6 N HCl and the precipitate was extracted into EtOAc (20 ml × 3). The combined organic extracts were washed with 0.1 N HCl, H_2O, dried over Na_2SO_4, and concentrated to give a residue that was recrystallized using a suitable solvent. Yield: 84%.

1.7.1.6.4 Method D: Using Fmoc-N_3 [61a]



mp. 219-220 °C;
$[\alpha]^{25}_D$ = -17.65 (*c* 1, DMF)

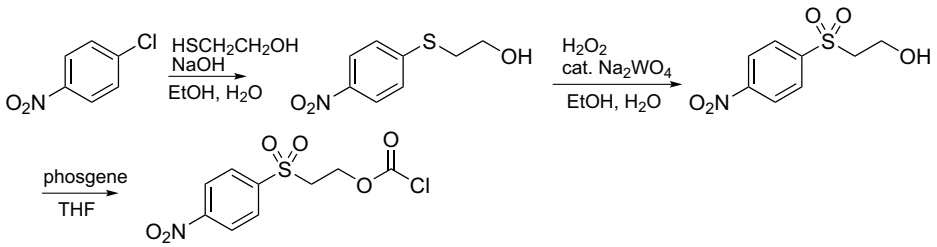
NaN_3 (0.78 g, 12.0 mmol) in H_2O (4 ml) was added to a solution of Fmoc-Cl (2.58 g, 10.0 mmol) in dioxane (5 ml) and the mixture was stirred at room temperature until completion (about 1 h, HPLC) while no precipitate appeared. Gln (1.6 g, 11.0 mmol), dissolved in 1% aqueous Na_2CO_3/dioxane (60 ml), was added to this mixture and it was stirred at room temperature for 48 h, during which the pH was kept between 8.0 and 10.0 by the addition of 10% aqueous Na_2CO_3 solution. Once the reaction was complete (HPLC), the mixture was poured into H_2O (100 ml) (keeping a basic pH) and extracted four times with *tert*-butyl methyl ether. The aqueous layer was cooled to 4 °C and acidified to pH 2.0 with 2 N HCl. The white precipitate was filtered, washed with ice H_2O (made slightly acidic with dilute HCl, pH 4–5), and dried overnight under vacuum. Yield: 71%.

Note: Fmoc-N_3 can also be prepared as an isolable solid (melting point 83–85 °C) through the treatment of Fmoc-Cl with NaN_3 in a water/acetone system and then used to introduce the Fmoc group [58b].

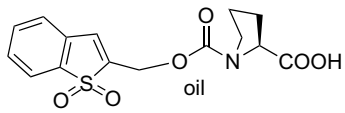
1.7.1.7 General Procedure for the Preparation of N^{α}-Nsc-Amino Acids [83]

To a solution of the amino acid (20.0 mmol) in DCM (40 ml) was added TEA (5.56 ml, 40.0 mmol) and TMS-Cl (5.27 ml, 40.0 mmol) dropwise with stirring. The mixture was refluxed for 1.5 h, cooled (0 °C) and Nsc-Cl (17 mmol) was added. After stirring at 0 °C for 30 min and at room temperature for 2 h, the solution was concentrated, and the residue was partitioned between Et_2O and 5% $NaHCO_3$ solution. The aqueous layer was acidified to pH 2.0–2.5 with 1 M H_2SO_4, extracted with EtOAc, dried (Na_2SO_4), and concentrated. The residue was recrystallized from EtOAc/petroleum ether.

Note: The synthesis of the reagent Nsc-Cl is outlined in the following scheme [262].



1.7.1.8 General Procedure for the Preparation of N^{α}-Bsmoc-Amino Acids [84a]



A solution of benzothiophenesulfone-2-methyl *N*-succinimidyl carbonate (0.5 g, 1.48 mmol) in acetone (10 ml) was added to the solution of Pro (0.17 g, 1.48 mmol) and $NaHCO_3$ (0.25 g, 2.96 mmol) in H_2O (10 ml), and the mixture was stirred at room temperature overnight. The mixture was diluted with H_2O and extracted twice with DCM. The aqueous layer was cooled and acidified to pH 2.0 with concentrated HCl.

The resulting white precipitate or oil was extracted with EtOAc (25 ml × 3), and the combined organic layers were washed with H_2O (30 ml), brine (30 ml), and dried over $MgSO_4$. The solvent was evaporated and the crude product was recrystallized. Yield: 90%.

1.7.1.9 General Procedure for the Preparation of N^{α}-Aloc-Amino Acids [88]

Aloc-Cl (10.6 ml, 0.1 mol) and 4 M NaOH (25 ml) were added alternatively within approximately 30 min to a solution of the amino acid (0.1 mol) in 4 M NaOH (25 ml) at 0 °C and the mixture was stirred for an additional 15 min at room temperature. The mixture was extracted with Et_2O and the aqueous layer was acidified with concentrated HCl. After cooling for several hours, the product was collected, dried, and recrystallized.

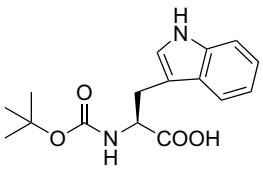
The DCHA salts were obtained by adding DCHA (1 equiv.) to a solution of the Aloc-protected amino acid derivatives in EtOH or EtOAc and precipitation with Et_2O or Et_2O/petroleum ether [92].

1.7.1.10 General Procedures for the Preparation of N^{α}-Boc-Amino Acids

1.7.1.10.1 Method A: Using $(Boc)_2O$ [99] To an ice-cold solution of the amino acid (10.0 mmol) in a mixture of dioxane (20 ml), H_2O (10 ml) and 1 N NaOH was added $(Boc)_2O$ (2.4 g, 11.0 mmol), and the mixture was stirred at room temperature for 30 min. The mixture was concentrated *in vacuo* to about 10–15 ml, cooled, covered with a layer of EtOAc (30 ml), and acidified to pH 2.0–3.0 with a dilute solution of $KHSO_4$ (to Congo paper red). The aqueous phase was extracted with EtOAc (15 ml × 2). The organic layers were combined, washed with H_2O (30 ml × 2), dried over Na_2SO_4, and evaporated. The residue was recrystallized with a suitable solvent.

Note: $(Boc)_2O$ is prepared by treating *tert*-butyl potassium carbonate (potassium *tert* butoxide and CO_2) with phosgene and then the resulting tricarbonate with diazabicyclo[2.2.2]octane [99a].

1.7.1.10.2 Method B: Using Boc-ON [100]



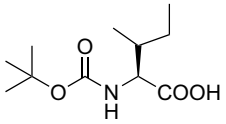
mp. 137-138 °C;
$[\alpha]^{25}_D$ = -20 (*c* 1, DMF)

Boc-ON (2.71 g, 11.0 mmol) and dioxane (6 ml) were added to a solution of Trp (2.05 g, 10.0 mmol) and TEA (2.1 ml, 15.0 mmol) in H_2O (6 ml) at room temperature, and stirred for an additional 2 h. After addition of H_2O (15 ml) and EtOAc (20 ml), the aqueous layer was separated, washed with EtOAc (20 ml), acidified with 5% citric acid solution, and extracted with EtOAc. The organic extracts were combined and concentrated. Yield: 99%.

Note: Preparation of Boc-ON:

- BzlCN and methyl nitrite are reacted in the presence of NaOH in MeOH.
- The resulting oxime is treated with phosgene in benzene and further with *tert*-butanol [100].

1.7.1.10.3 Method C: Using Boc-N_3 [263–265]



mp. 65-68 °C;
$[\alpha]^{20}_D$ = +3.8 (*c* 10, 80% AcOH)

i) Generation of *tert*-butyl azidoformate (Boc-N_3). To an ice-cold solution of Boc-$NHNH_2$ (477.0 g, 3.6 mmol) in dioxane (2 l) and H_2O (500 ml) was added slowly 5 M HCl (720 ml, 3.6 mmol) followed by a solution of $NaNO_2$ (255.0 g, 3.6 mmol) in H_2O (750 ml) and the mixture was stirred for 1–3 h at room temperature.

ii) Ile (393.0 g, 3.0 mol) was suspended in the above solution containing Boc-N_3, and dioxane (500 ml) was added and titrated at pH 10 with 4 M NaOH (1.5 l) over a period of 12 h. The mixture was neutralized to pH 7.0 with 1 M H_2SO_4 and concentrated. It was then acidified to pH 3–3.5 and extracted with EtOAc. The organic phase was washed with H_2O, dried (Na_2SO_4), and concentrated to an oily residue, which solidified under petroleum ether. Yield: 93%.

Caution: Warning Boc-N_3 is sensitive to heat and shock.

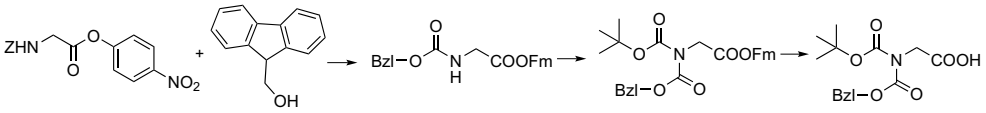
1.7.1.11 General Procedure for the Preparation of *N*,*N*′-di-Boc-Amino Acids [125]

i) $(Boc)_2O$ in MeCN (0.5 ml, 1.1 mmol) was added to a solution of the Boc-Gly-OBzl (0.26 g, 1.0 mmol) and DMAP (0.012 g, 0.1 mmol) in MeCN (1–2 ml), and the reaction was monitored by TLC (2: 1 toluene/MeCN or DCM). Upon completion of the reaction (If the starting material remained after 4–6 h, more $(Boc)_2O$ (approximately 0.5 equiv.) was added; if necessary, this procedure is repeated until all starting material is consumed.) the resulting mixture was concentrated (in some cases, excess $(Boc)_2O$ was removed under high vacuum overnight) and the residue was dissolved in EtOAc, washed with H_2O and dried. The solvent was evaporated and the product obtained was recrystallized from light petroleum. Yield: 99%; melting point 31–31.5 °C.

ii) The Boc_2-amino acid benzyl ester was hydrogenated for 1–2 h with 5% (w/w) of Pd/C in MeOH. The catalyst was filtered off and the filtrate was concentrated to afford a residue, which crystallized spontaneously when allowed to stand. The product was recrystallized from ether/light petroleum or EtOAc/light petroleum.

Note: *N*,*N*′-di-Boc-amino acids are also prepared starting from Boc-amino acid allyl esters [125].

N,N.Boc, Z-Gly-OH [125]



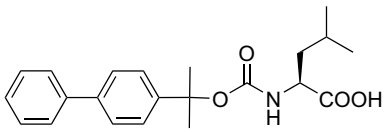
i) To a suspension of 9-fluorenylmethanol (2.15 g, 11.0 mmol) and imidazole (1.22 g, 18.0 mmol) in toluene, was added *o*-nitrophenyl ester of Z-Gly (2.09 g, 10.0 mmol) and the reaction was stirred at room temperature overnight. The solvent was removed, and the residue was taken up in EtOAc and washed with 1 M $KHSO_4$, 1 M Na_2CO_3, and saturated NaCl solutions. After drying, evaporation gave an oil that crystallized from EtOAc/light petroleum to obtain pure Z-Gly-OFm as an oil.

ii) Z-Gly-OFm (1.95 g, 5.0 mmol) and DMAP (61 mg, 0.5 mmol) were suspended in MeCN and Boc_2O (1.2 g, 6.0 mmol) was added, and the mixture was stirred for 3 h at room temperature. The solution was evaporated and the residue was dissolved in EtOAc. The organic phase was washed with H_2O and brine, dried, and evaporated to afford an oil that was chromatographed on a short column eluting first with toluene, then with toluene/MeCN (4: 1). Yield: 83%.

iii) *N,N′*-Boc, Z-Gly-OFm (1.0 g, 2.1 mmol) was treated with a 15% solution of piperidine in DMF (10 ml) for 2 h. The mixture was partitioned between EtOAc (100 ml) and 1 M $KHSO_4$ (30 ml). The organic phase was washed with further portions of 1 M $KHSO_4$ (30 ml × 2) and then extracted with 1 M $NaHCO_3$ (30 ml × 3). The combined aqueous extracts were acidified to pH 3.0 with solid $KHSO_4$ and extracted with EtOAc (30 ml × 3). The organic extract was washed with brine and dried ($MgSO_4$), and evaporated to afford a colorless oil. Yield: 64%.

Note: The above procedure gives good yields particularly for Gly. For other amino acids, acceptable yields can be obtained using allyl esters instead of Fm esters.

1.7.1.12 General Procedure for the Preparation of N^{α}-Bpoc-Amino Acids [266]



mp. 227-230 °C;
$[\alpha]^{22}_D$ = -12.2 (*c* 1, MeOH)

Leu (13.1 g, 100 mmol) was dissolved in a 2.5 *N*-benzyltrimethylammonium hydroxide in MeOH (40 ml, 100.0 mmol) and the solvent was removed *in vacuo*. The residue was dissolved in DMF (30 ml), the solution was evaporated, and the addition and evaporation of DMF was repeated. The dry salt was redissolved in DMF (40 ml), warmed to 50 °C, 2-(biphenyl-4-yl)-2-propyl-phenyl carbonate (33.3 g, 100 mmol) was added, and the mixture was stirred at 50 °C for 3 h and then cooled. The mixture was diluted with H_2O (200 ml) and ether (200 ml) was added. The

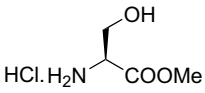
aqueous phase was acidified to pH 2.0–3.0 with a 1 M citric acid solution and re-extracted with ether (100 ml × 2). The combined ether extracts were washed with H_2O (100 ml × 2), dried over Na_2SO_4, and evaporated at 30 °C. The crystalline residue was triturated with ether (15 ml) and petroleum ether (boiling point 40–60 °C) and the insoluble product was collected by filtration. Yield: 70%.

Note: 2-Biphenyl-4-yl-2-propyl-phenyl carbonate is prepared by treating *p*-biphenylyl-dimethyl-carbinol with phenyl chlorocarbonate in DCM in the presence of pyridine [266].

1.7.1.13 General Procedures for the Preparation of Amino Acid Methyl Esters

1.7.1.13.1 Preparation of Amino Acid Methyl Ester Hydrochloride Salts

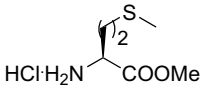
Method A: Using $SOCl_2$ [141]



mp. 168 °C;
$[\alpha]^{25}_D$ = -2.5 (*c* 1.8, DMF)

Freshly distilled $SOCl_2$ (26 ml) was added (over 10 min) to absolute MeOH (20 ml) maintained at −10 °C followed by the addition of Ser (10.5 g, 10.0 mmol). The suspension was stirred at room temperature until complete dissolution. After 24 h, the solvent was removed and the residue was recrystallized from MeOH/Et_2O. Yield: 99%.

Method B: Using 2,2-Dimethoxypropane and Aqueous HCl [267]

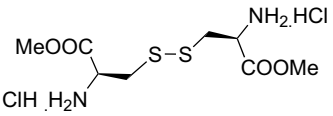


mp. 147-150 °C;
$[\alpha]^{26}_D$ = +25.2 (*c* 5.1, H_2O)

A solution of 36% HCl (1 ml) was added to a suspension of Met (0.199 g, 1.0 mmol) and 2,2-dimethoxypropane (10–15 ml), and the reaction mixture was allowed to stand at room temperature for 18 h. The mixture that darkened considerably after standing was concentrated and the residue was dissolved in a minimum amount of absolute MeOH. Addition of about 25 ml of Et_2O resulted in solidification of the product, which was recrystallized from MeOH-ether. Yield: 95%.

Note: In the case of Lys and of Glu, addition of 3–4 ml of MeOH and refluxing for 2–5 h is recommended.

Method C: Using TMS-Cl [268]

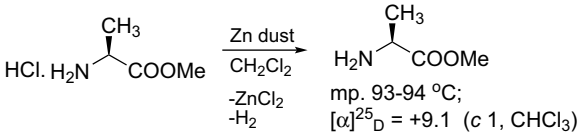


mp. 173 °C dec;
$[\alpha]^{26}_D$ = +37 (*c* 0.013, MeOH)

Freshly distilled TMS-Cl (26.0 ml, 0.2 mol) was added slowly to cystine (24 g, 0.1 mol) with stirring. MeOH (100 ml) was added and stirring was continued at room temperature. The mixture was concentrated to obtain the product. Yield: 98%.

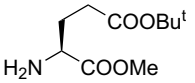
Note: Amino acid methyl ester TsOH salts are prepared by refluxing the mixture of amino acid and TsOH in MeOH for 24 h [269].

1.7.1.13.2 Isolation of Amino Acid Methyl Esters: Deprotonation of the Hydrochloride Salt Using Zinc Dust [142]



Activated zinc dust (100 mg) was added to a suspension of H-Ala-OMe·HCl (0.103 g, 1.0 mmol) in DCM or THF (10 ml) and the mixture was stirred for 5 min at room temperature. The mixture was filtered and the filtrate was evaporated. The product was precipitated using Et_2O. Yield: quantitative.

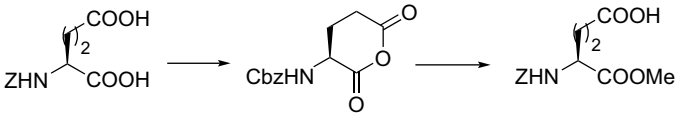
1.7.1.13.3 Glutamic Acid α-Methyl, γ-*tert*-Butyl Diester Using Diazomethane [270]



mp. 135-136 °C dec;
$[\alpha]^{20}_D$ = +17 (*c* 0.03, MeOH)

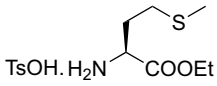
A solution of CH_2N_2 in ether was added to a solution of Glu(O*t*Bu) (5.0 g, 24.6 mmol) in MeOH (100 ml) at 0 ° to + 5 °C to give a stable yellow-colored solution. The mixture was kept for 6 h at 20–22 °C, several drops of glacial AcOH were added, and the solution was evaporated to dryness. The residue was dissolved in EtOAc (50 ml), and the organic phase was washed with 1 N $NaHCO_3$ (50 ml × 3), H_2O, dried ($MgSO_4$), and evaporated to obtain the residue that was recrystallized from EtOAc/hexane. Yield: 91%.

1.7.1.13.4 Z-Glu-OMe via Methanolysis of Cyclic Anhydride [271]



Z-Glu was self-condensed in the presence of Ac_2O at 20 °C and the resulting anhydride was treated with MeOH/DCHA/Et_2O at 20 °C for 12 h. Yield: 55%.

1.7.1.14 General Procedure for the Preparation of Amino Acid Ethyl Esters ([1f], p. 30)

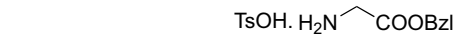


mp. 124-126 °C;
$[\alpha]^{22}_D$ = +13 (*c* 3.3, 95% EtOH)

TsOH (monohydrate, 3.8 g, 20.0 mmol) was added to a suspension of Met (1.49 g, 10.0 mmol) in absolute EtOH (50 ml) and the mixture was refluxed for 24 h. The solvent was evaporated and the residue was triturated with dry Et_2O (100 ml). The product was washed with ether (100 ml) and dried *in vacuo* over P_2O_5. Yield: 98%.

Note: The procedure is a modification of the method reported for the synthesis of amino acid benzyl esters [272].

1.7.1.15 General Procedure for the Preparation of Amino Acid Benzyl Ester *p*-Toluenesulfonate Salts [158]

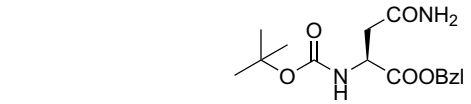


mp. 132-134 °C

Gly (18.8 g, 250.0 mmol) and TsOH (monohydrate, 48.5 g, 255 mmol) were added to a mixture of freshly distilled benzyl alcohol (100 ml) and C_6H_6 (50 ml), and the mixture was refluxed in a Dean–Stark apparatus. When no more H_2O appeared in the distillate the mixture was allowed to cool to room temperature, diluted with ether (500 ml), and cooled for an additional 2 h. The crystals formed were collected, washed with ether (200 ml), and dried in the air. The product was recrystallized from MeOH-ether. Yield: 90%.

1.7.1.15.1 **Preparation of Amino Acid Benzyl Ester *p*-Toluenesulfonate Salts Under Microwave Irradiation [159]** A mixture of the amino acid (10.0 mmol), benzyl alcohol (3 ml), and TsOH (2.09 g, 11.0 mmol) in a 100 ml glass beaker and exposed to microwaves (domestic microwave oven; LG Little Chef model 194A; 2450 MHz) operating at its 40% power for 50–60 s. After the completion of reaction, the mixture was cooled to room temperature, ether was added, and the precipitate was collected and washed with ether (25 ml × 2). Yield: H-GlyOBzl·HCl: 92%; H-AlaOBzl·HCl: 93%; H-ValOBzl·HCl: 94%; H-PheOBzl·HCl: 95%.

Boc-Asn-OBzl through Benzylation of Cs Salt [161]



mp. 120-122 °C;
$[\alpha]^{25}_D$ = -17.3 (*c* 1, DMF)

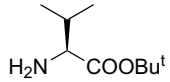
i) A solution of Boc-Asn (23.2 g, 100 mmol), H_2O (40 ml), and MeOH (400 ml) was neutralized with 20% Cs_2CO_3 in H_2O, and then evaporated to dryness. DMF (250 ml) was added and removed *in vacuo* at 45 °C. Addition and evaporation of DMF was repeated.

ii) The cesium salt of Boc-Asn obtained from the above step was treated with DMF (250 ml) and BzlBr (18.8 g, 110 mmol) at room temperature for 6 h, evaporated to dryness, and the residue was triturated with H_2O (1 l). The solid was dissolved in EtOAc (300 ml). The organic layer was washed with H_2O

(150 ml), dried over Na_2SO_4, and evaporated. The product was recrystallized from EtOAc/hexane. Yield: 90%.

Note: The procedure yields methyl, Trt, and phenacyl esters also [161].

1.7.1.16 General Procedure for the Preparation of tert-Butyl Esters of N^{α}-Unprotected Amino Acids Using Isobutene [168]

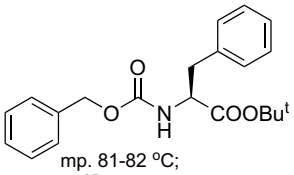


mp. 147-149 °C;
$[\alpha]^{25}_D$ = +20.5 (*c* 2, EtOH)

Liquid isobutene (25 ml) was added to a solution of Val (1.52 g, 0.013 mol) in dioxane (25 ml) and concentrated H_2SO_4 (2.5 ml) in a 500 ml pressure bottle, and the mixture was stirred at room temperature for 4 h. The solution was poured immediately into a cold mixture of ether (200 ml) and 1 N NaOH (125 ml), and the aqueous phase was washed with ether. The ether solution was dried over sodium sulfate and concentrated to about 5 ml. This was diluted with ether (25 ml). Addition of dry HCl gave the crystalline hydrochloride, which was recrystallized from EtOAc. Yield: 65%.

Note: *tert*-Butyl esters of N^{α}-unprotected amino acids can also be accessed via a two-step procedure in which Z-amino acids are esterified with isobutene followed by catalytic hydrogenation of the Z group [161].

1.7.1.16.1 Preparation of Z-Phe-O*t*Bu by the Silver Salt Method [170]

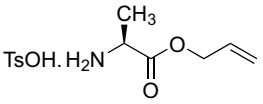


mp. 81-82 °C;
$[\alpha]^{25}_D$ = -4.4 (c 2, EtOH)

i) Z-Phe (54.5 g, 182 mmol) was dissolved in an excess of concentrated $(NH_4)OH$, and the solution was concentrated *in vacuo* to near dryness. *iso*-Propanol was added and the concentrating process was repeated. H_2O (200 ml) was added and the resulting solution was combined with a solution of $AgNO_3$ (30.9 g in 200 ml of H_2O). The resulting precipitate of silver Z-phenylalaninate was collected and dried. Yield: 88%; melting point 183–188 °C dec.

ii) *tert*-Butyl iodide was slowly added to a suspension of silver Z-phenylalaninate in ether (500 ml). The precipitate of AgI was removed and washed with dry ether. Evaporation of the combined ether portions in an open dish left as a crystalline solid that was recrystallized by making a solution of it in warm MeOH (100 ml) and adding this to H_2O (100 ml) containing sodium bisulfite (2.0 g). Yield: 33% from the silver salt.

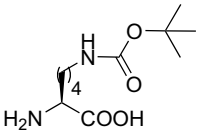
H-Ala-OAl·TsOH[176c]



mp. 84-85 °C;
$[\alpha]^{25}_D$ = -1.6 (*c* 1, MeOH)

A mixture of Ala (2.2 g, 25.0 mmol), allyl alcohol (15.0 g, 0.25 mmol), and $TsOH·H_2O$ (5.7 g, 30.0 mmol) in benzene (200 ml) was refluxed in a Dean–Stark apparatus until the requisite amount of H_2O was trapped. The benzene was evaporated and the product was precipitated by the addition of Et_2O. Yield: 98%.

Lys(Boc) [185, 273]



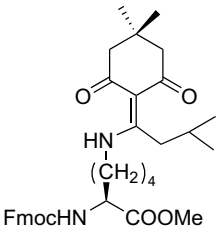
mp. 63-64.5 °C;
$[\alpha]^{20}_D$ = -6.97 (*c* 1, MeOH)

i) A solution of $CuSO_4·5H_2O$ (3.12 g, 12.5 mmol) in H_2O (25 ml) was added to a stirred solution of Lys·HCl (4.56 g, 25.0 mmol) in 2 M aqueous $NaHCO_3$ (25 ml). Then $NaHCO_3$ (2.1 g, 50.0 mmol) and 96% $(Boc)_2O$ (7.37 g, 32.5 mmol) in acetone (30 ml) were added. The mixture was stirred for 24 h, MeOH (6.5 ml) was added, and the solution was stirred for a further 12 h. H_2O (25 ml) and EtOAc (25 ml) were added, and the precipitate was filtered off. The precipitate was suspended in H_2O (62.5 ml) and filtered. These operations were repeated twice and a light-blue solid was obtained that was air-dried. Yield: 94%.

ii) To a suspension of $[Lys(Boc)]_2Cu$ (13.85 g, 25.0 mmol) in H_2O (500 ml), 8-quinolinol (9.45 g, 65.0 mmol) was added and the mixture was stirred for 5 h. Cu(II)8-quinolinolate was filtered off and washed with H_2O (25 ml). The precipitate was suspended in H_2O (250 ml) and kept for several hours. It was filtered off and washed with H_2O. All filtrates and washings were extracted with EtOAc (150 ml × 2), and the organic phase was evaporated. Yield: 96%.

Note: Decomposition of the copper complex is carried out with H_2S, EDTA, and Na_2S also [274].

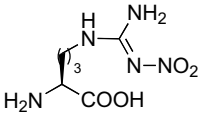
Lys(ivDde) [194a, 275]



mp. 69-71 °C

TFA (0.19 ml, 2.5 mmol) was added to a suspension of Fmoc-Lys-OH (9.2 g, 25.0 mmol) and 2-(1-hydroxy-3-methylbutylidene)-5,5-dimethylcyclo-hexane-1,3-dione (11.2 g, 50.0 mmol) in EtOH (192 ml) at room temperature and the mixture was refluxed for 60 h. The solvent was evaporated and the residue was dissolved in EtOAc (650 ml). The organic phase was washed with 1 M $KHSO_4$ (50 ml × 2), dried ($MgSO_4$), and concentrated to afford a yellow oil which was triturated with hexane and crystallized from EtOAc/hexane. Yield: 64%.

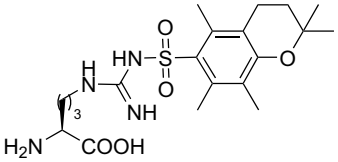
Arg(NO_2) [276]



mp. 251-252 °C;
$[\alpha]^{25}_D$ = +24.3 (*c* 4.12, 2 N HCl)

Arg free base (4.3 g, 25.0 mmol) was added slowly with stirring to a mixture of fuming HNO_3 (5.75 ml) and fuming H_2SO_4 (3.75 ml, containing 30% SO_3) maintained in an ice salt-bath. The mixture was stirred for 1 h with cooling and then poured onto crushed ice (about 50 g). The pH was adjusted to 8.0–9.0 with concentrated NH_4OH and then readjusted to pH 6.0 with glacial AcOH, and the solution was kept in a refrigerator for 4 h. The precipitate was collected, and recrystallized from hot H_2O, washed with 95% EtOH and ether, and dried. Yield: 82%.

Arg(Pbf) [19, 277]

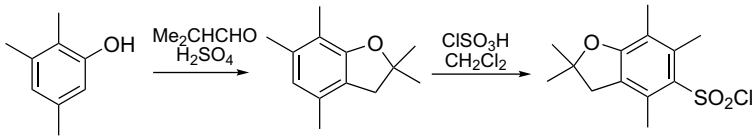


Pbf-Cl (11.45 g, 40.0 mmol) in acetone (55 ml) was added to a solution (at 0 °C) of Z-Arg-OH (7.5 g, 25.0 mmol) in 3.2 M NaOH (32.5 ml) and acetone (88.5 ml), and stirred at 0 °C for 2 h and for a further 2 h at room temperature. After acidification to pH 6.5 with saturated citric acid solution, the acetone was evaporated and the remaining solution was further acidified to pH 3 with saturated citric acid solution, diluted with H_2O (110 ml), and extracted with EtOAc (110 ml × 3). The combined extracts were filtered, washed with H_2O (150 ml × 2) and brine (150 ml × 2), and dried over $MgSO_4$. The solution was then concentrated, cooled in an ice bath, and cyclohexylamine (CHA; 2.85 ml, 25 mmol) was added. Addition of anhydrous ether gave a thick white gum, which solidified on standing overnight at 4 °C. Recrystallization from MeOH/Et_2O afforded the product.

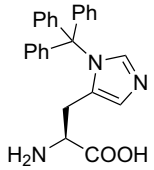
Z-Arg(Pbf)-OH·CHA was converted to its free acid and then subjected to hydrogenation via catalytic hydrogenation with 10% palladium on charcoal in MeOH under H_2 atmosphere [277].

Note:

- The above procedure is a modification of the procedure employed for the synthesis of Arg(Pmc). The Pmc-Cl in the latter is replaced by Pbf-Cl [277b].
- The preparation of Pmc-Cl is outlined in the following scheme. Step 1 is a modified procedure for the synthesis of the benzofuran nucleus [277c].



H-His(N^{τ}-Trt)-OH [25]

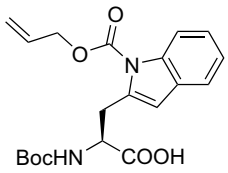


mp. 220-222 °C;
$[\alpha]^{25}_{D}$ = -2.1 (1:1 THF:H_2O)

Me_2SiCl_2 (1.21 ml, 10.0 mmol) was added to a suspension of His (1.55 g, 10.0 mmol) in DCM (15 ml) and the mixture was refluxed for 4 h. Then, Et_3N (2.79 ml, 20.0 mmol) was added and reflux was continued for an additional 15 min. This was followed by the addition of Et_3N (1.39 ml, 10.0 mmol) and a solution of Trt-Cl (2.79 g, 10.0 mmol) in DCM (10 ml) under stirring at room temperature. After 2 h, an excess of MeOH was added and the solvent was evaporated. H_2O was added to the residue and the pH was adjusted to 8.0–8.5 by dropwise addition of Et_3N. The resulting slurry was shaken well with $CHC1_3$ and the insoluble material was collected. Further washing with H_2O and Et_2O provided the product that was recrystallized from THF/H_2O (1:l). Yield: 97%. IR: 3550, 2200, 1650, 1560, 750, 700 cm^{-1}.

Note: The N^{α},N^{τ}-Trt$_2$-His (melting point 198 °C; $[\alpha]_D{}^{25} = +3.6$ (*c* 5, pyridine)) can be analogously prepared using 2 equiv. of Trt-Cl [25].

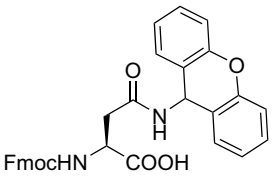
Boc-Trp(Aloc)-OH [213]



mp. 123-124 °C dec;
$[\alpha]^{25}_{D}$ = -16.7 (*c* 1, DMF)

Aloc-Cl (2.75 ml, 26.0 mmol), DBU (3.87 ml, 26.0 mmol), and DMAP (244 mg, 2.0 mmol) were added to a solution of Boc-Trp-O*t*Bu (7.95 g, 22.0 mmol) in MeCN (50 ml) and the mixture was stirred at 25 °C for 5 h. The solvent was evaporated and the residue was dissolved in EtOAc. The organic phase was washed with H_2O, saturated $NaHCO_3$, 5% $KHSO_4$/10% K_2SO_4, brine, dried over Na_2SO_4, and concentrated to afford Boc-Trp(Aloc)-O*t*Bu, which was recrystallized from Et_2O/hexane. Yield: 85%. The ester from the above step was dissolved in a mixture of TFA (6.0 ml) and H_2O (0.12 ml), and the solution was stirred for 30 min under N_2. The solvent was removed and the residue was concentrated 3 times from toluene, and the residue was suspended in a mixture of dioxane (12 ml) and H_2O (12 ml), and then treated with $(Boc)_2O$ (0.75 g, 3.4 mmol) in the presence of $NaHCO_3$ (1.2 g, 13.6 mmol) at 25 °C for 18 h. The resulting solution was diluted with EtOAc and acidified to pH 2 with 1 M HCl. The organic phase was washed with 5% $KHSO_4$/10% K_2SO_4, brine, dried over Na_2SO_4, and concentrated. The product was crystallized from Et_2O/hexane. Yield: 82%.

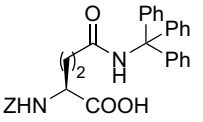
Fmoc-Asn(Xan)-OH [278]



mp. 206-209 °C

To a solution of Fmoc-Asn-OH (8.85 g, 25.0 mmol) and 9*H*-xanthen-9-ol (6.2 g, 37.5 mmol) in DMF (175 ml) was added TFA (8.75 ml, 111.5 mmol) in small portions, and the mixture was stirred at room temperature for 4 h, then poured into ice-H_2O (2 l). The precipitate was collected and washed with H_2O, ice-cold MeOH, and Et_2O. Yield: 99%.

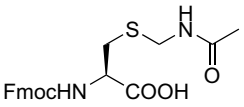
Z-Gln-(Trt)-OH [214]



mp. 161-162 °C;
$[\alpha]^{25}_D$ = -45 (MeOH)

A suspension of Z-Gln-OH (6.97 g, 25.0 mmol), Trt-OH (12.99 g, 50.0 mmol), Ac_2O (4.72 ml, 50.0 mmol), and concentrated H_2SO_4 (0.12 ml, 1.12 mmol) in AcOH (75 ml) was stirred for 1 h at 50 °C. The solution was then slowly added to cold H_2O (750 ml), and precipitate was collected and dissolved in EtOAc (50 ml). The organic layer was washed with H_2O, dried, and concentrated to leave a residue, which was crystallized from EtOAc/hexane. Yield: 67%.

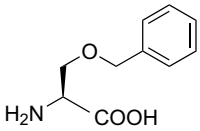
Fmoc-Cys(Acm)-OH [279]



mp. 147-148 °C;
$[\alpha]^{25}_D$ = -44 (*c* 1, DMF)

To a solution of Cys (3.95 g, 25.0 mmol) and *N*-(hydroxymethyl)acetamide (3.11 g, 30.2 mmol) in H_2O (5.82 ml) at 0 °C was added a mixture of TfOH/TFA (1: 19, 37 ml) and the mixture was stirred for 90 min under argon. The solvent was removed and the residue was reconcentrated from Et_2O (20 ml × 5). The resulting oil was dissolved in 10% aqueous Na_2CO_3 (50 ml) and the pH was adjusted to 10.0 with 10% aqueous Na_2CO_3. To this, Fmoc-N_3 (6.02 g, 22 mmol) in dioxane (65 ml) was added, and kept in an ice bath for 2 h and at room temperature for 48 h. The mixture was diluted with H_2O and extracted with Et_2O. The aqueous phase was acidified and extracted with EtOAc, and the organic layer was dried with $MgSO_4$ and concentrated. The product was crystallized from DCM/hexane. Yield: 62%.

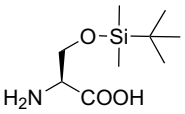
Ser(Bzl) [230]



mp. 221-222 °C;
$[\alpha]^{25}_D$ = +6.0 (*c* 1, 3M HCl)

To H-Ser-OLi (0.81 g, 10.0 mmol) suspended in THF (15 ml) was added $BF_3 \cdot OEt_2$ (6.0 ml) and the solution was stirred at room temperature for 6 h, and then at 40–45 °C for an additional 2 h to give 2,2-difluoro-4-(hydroxymethyl)-1,2,3-oxazaborolidin-5-one in quantitative yield. The THF was evaporated and the residue was dissolved in dioxane (30 ml), and then treated with benzyl trichloroacetimidate (2.15 ml, 11.5 mmol). After 2 h, the mixture was treated with anhydrous MeOH (5 ml), stirred for 10 min, and then heated with 1 M NaOH (30 ml); after stirring for 30 min and concentration at reduced pressure, the residue was dissolved in H_2O (200 ml) and the resulting solution was washed with Et_2O (15 ml × 3). The aqueous phase was adjusted to pH 6.0 and applied to an Amberlite XAD-4 resin column. The column was washed with H_2O and then with 50% EtOH. The latter eluant was collected and concentrated. Yield: 95%.

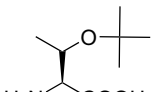
H-Ser(TBDMS)-OH [280]



Imidazole (13.6 g, 2.0 equiv.) and TBDMS-Cl (20.46 ml, 1.1 equiv.) were added to Ser (10.6 g, 0.1 mmol) in DMF (100 ml) and the mixture was stirred at room temperature for 20 h. The DMF was evaporated to give an oily residue which was stirred with H_2O/hexane (1: 1) for 4 h to obtain a white solid. The latter was collected, washed with hexane, and air-dried. Yield: 91%.

Note: Z-Ser(TBDMS)-OH is prepared analogously starting from Z-Ser [276].

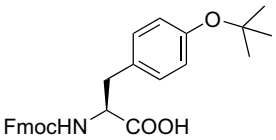
Thr(tBu) [281]



mp. 259-260 °C;
$[\alpha]^{20}_D$ = -42.1 (*c* 2, MeOH)

To a solution of *p*-nitrobenzyl ester of Z-Thr (38.8 g, 100 mmol) in DCM (400 ml) kept in an ice-water bath, isobutene (350 ml) and concentrated H_2SO_4 (5 ml) were added with caution, and left at room temperature for 4 days. The mixture was cooled again to about 0 °C, washed with an ice-cold 5% solution of Na_2CO_3 in H_2O (200 ml × 3), the aqueous phases were re-extracted with DCM (100 ml × 2), and the organic phases were combined washed with H_2O until the washes were neutral. The solution was dried over P_2O_5 and evaporated *in vacuo*. The yellow, crystalline residue was dissolved in a small volume of EtOAc and diluted with hexane until some dark oil separates. The clear solution was decanted and further diluted with hexane. The *tert*-butyl ether separated as needles. Yield: 80%. melting point 55–56.5 °C. The latter (22.3 g, 50.0 mmol) was dissolved in MeOH (150 ml), the solution was diluted with H_2O (50 ml) and AcOH (4 ml), the air was displaced with N_2, a 10% palladium on charcoal catalyst (4.5 g) was added, and the mixture was hydrogenated at room temperature. After removal of the catalyst by filtration and the solvent by evaporation *in vacuo*, the residue was triturated with EtOH and recrystallized from EtOH/acetone. Yield: 85%.

Fmoc-Tyr (tBu)-OH [282]



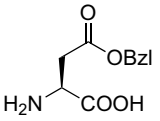
mp. 150-151 °C;
$[\alpha]^{20}_D$ = -28.0 (*c* 1, DMF)

i) Fmoc-Tyr-OMe (5.0 g, 12.0 mmol), concentrated H_2SO_4 (0.33 ml, 6.0 mmol), and DCM (100 ml) were stirred under isobutene gas (260 Torr) for 6 h at room temperature. The solution was washed with cold 10% $NaHCO_3$ (100 ml × 2) and brine (100 ml), dried (Na_2SO_4), and concentrated. The residue was dissolved in MeOH/CCl_4 (1: 1, 400 ml), washed with H_2O (300 ml), MeOH/H_2O (1: 1, 200 ml × 2), dried (Na_2SO_4), and the solvent was evaporated to give a

white solid that was recrystallized (DCM/hexane). Yield: 83%; melting point 90–92 °C; $[\alpha]_D^{20} = -22.1$ (*c* 1, DMF).

ii) A mixture of Fmoc-Tyr(*t*Bu)-OMe (2.0 g, 4.22 mmol) in MeCN (250 ml) and 3% Na_2CO_3 (375 ml) was stirred for 15 h, then washed with hexane (500 ml × 3), acidified with 2 M HCl to pH 3–4, and extracted with $CHCl_3$ (600 ml × 2). The combined $CHCl_3$ fractions were washed with brine (500 ml), dried (Na_2SO_4), and the solvent was removed to provide an oil that gave white crystals from EtOAc/hexane. Yield: 74%.

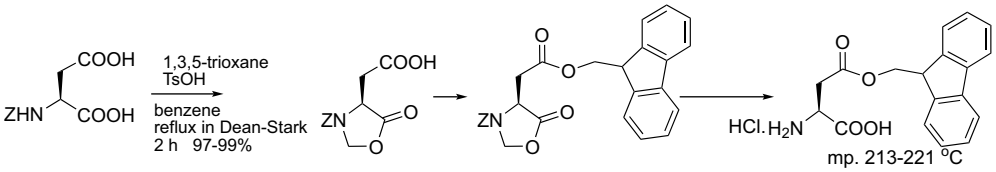
Asp(OBzl) [239]



mp. 218-220 °C;
$[\alpha]^{25}_D$ = +28 (*c* 1, 1 N HCl)

Freshly distilled benzyl alcohol (100 ml) was added to a mixture of dry Et_2O (100 ml) and concentrated H_2SO_4 (10 ml). The ether was evaporated and finely ground Asp (13.4 g, 100 mmol) was added in small portions with stirring. The resulting solution was kept at room temperature for about 1 day, diluted with 95% EtOH (200 ml), and neutralized by the dropwise addition of pyridine (50 ml) under stirring. The mixture was stored in the refrigerator and washed by trituration on the filter with ether. The product was recrystallized from hot H_2O containing a few drops of pyridine. Yield: 40%.

Asp(Fm) [242b]

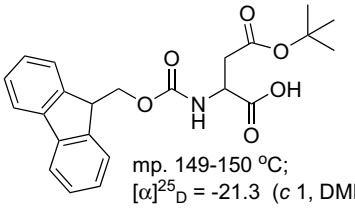


i) Esterification of carboxy group of oxazolidin-5-one of Z-Asp. The title oxazolidin-5-one (2.06 g, 10.0 mmol) and DCC (2.06 g, 10 mmol) were dissolved in dry THF (150 ml) and stirred at room temperature. A second solution, made by dissolving 9-fluorenylmethanol (1.37 g, 7.0 mmol) and DMAP (0.12 g, 1.0 mmol) in dry THF (20 ml), was added dropwise to the first solution over a period of 20–25 min. The mixture was stirred at room temperature for 1 h (TLC; $CHCl_3$/MeOH/AcOH 94: 5: 1 or $CHCl_3$/MeOH/AcOH 80: 18: 2). Additional DCC (approx. 2.0 mmol) was added every 30 min until all the 9-fluorenylmethanol was consumed. The solution was filtered, and

the THF was evaporated and replaced with an equal volume of EtOAc. The solution was placed in the freezer for about 1 h and any insoluble dicyclohexylurea (DCU) found was filtered off. This step was repeated with a fresh aliquot of EtOAc. The EtOAc solution was washed with H_2O (twice), 5% $NaHCO_3$, H_2O, 5% HCl, and H_2O. The organic phase was dried over anhydrous $MgSO_4$ and concentrated to afford a pale yellow, viscous oil. Yield: 84–87%.

ii) Concomitant removal of N^{α}-Z group and ring opening of 5-oxazolidinone. The above product (4.57 g, 10.0 mmol) was dissolved in neat TFA (50-60 ml). HBr gas was bubbled through this stirred solution for 10–15 min and then it was stirred at room temperature for another 30 min. Upon completion of reaction (TLC), HBr/TFA was removed *in vacuo* to afford a deep-blue oil. The oil was dissolved in glacial AcOH (50–60 ml) and lyophilized to complete dryness. This residue was dispersed in EtOAc (150 ml) and HCl gas was bubbled through the slurry with stirring in order to generate the less-soluble HCl salt of the amino acid. The pale yellow precipitate that formed was filtered, washed several times with EtOAc and dried under vacuum. Yield: 72–78%.

Fmoc-Asp (OtBu)-OH [240]



mp. 149-150 °C;
$[\alpha]^{25}_D$ = -21.3 (*c* 1, DMF)

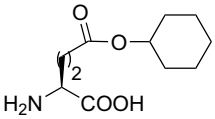
A mixture of Asp (3.9 g, 29.0 mmol) and TsOH (11.1 g, 58.0 mmol) in dioxane (140 ml) was stirred under isobutene gas (350 mbar) for 3 days at room temperature. To this was added aqueous Na_2CO_3 (175 ml, 10%, w/v) followed by dropwise addition of Fmoc-OSu (9.9 g, 29.0 mmol) in dioxane (50 ml) at 0 °C. After stirring overnight at room temperature, the mixture was poured into ice-H_2O (300 ml) and washed with Et_2O (300 ml × 3) to remove excess Fmoc-OSu and Fmoc-di-*tert*-butyl-Asp. The aqueous phase was chilled (0 °C), acidified to pH 5.5 with 1 N HCl, and extracted with EtOAc (300 ml × 3). The combined EtOAc fractions were washed with brine, dried ($MgSO_4$) and concentrated to yield a white solid. This is a mixture of mono-*tert*-butyl esters (Fmoc-Asp(O*t*Bu)-OH and Fmoc-Asp-O*t*Bu) that was dissolved in DCM/petroleum ether (1:1, boiling point 30–60 °C) and chilled (0 °C) overnight. The product was obtained as crystals which were filtered and washed with petroleum ether. Overall yield 35% (HPLC purity 99.5%, C_{18} column, 60% MeOH in 0.1% TFA).

Note:

- The α-*tert*-butyl ester can be crystallized as its DCHA salt from a DCM solution.
- Fmoc-Glu(O*t*Bu)-OH is prepared analogously. Once the mixture of mono-*tert*-butyl esters are obtained, it is dissolved in Et_2O and the desired product was crystallized as the DCHA salt by the addition of DCHA (2.5 ml, 0.6 equiv). HPLC

was conducted via the same solvent system. Overall yield: 39%; melting point 78–80 °C; $[\alpha]_D^{25} = -15.7$ (*c* 1, DMF).

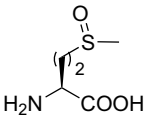
H-Glu(OCγ)-OH [283]



mp. 195-198 °C

Cyclohexanol (250 ml, 2.5 mol) and Glu (36.7 g, 0.25 mol) were added under stirring to chilled Et_2O (250 ml) containing H_2SO_4 (25 ml, 0.5 mmol), and the suspension was heated in a rotary evaporator at 70 °C for 2 h under reduced pressure. The bulk of the solvent was removed during this procedure. The resulting oil was partitioned between EtOAc (250 ml) and 5% aqueous $KHCO_3$ (300 ml). The pH was adjusted to 7.0 with 4 M NaOH and the aqueous layer was concentrated until precipitation occurred. The resulting suspension was chilled overnight and filtered. Yield: 49%.

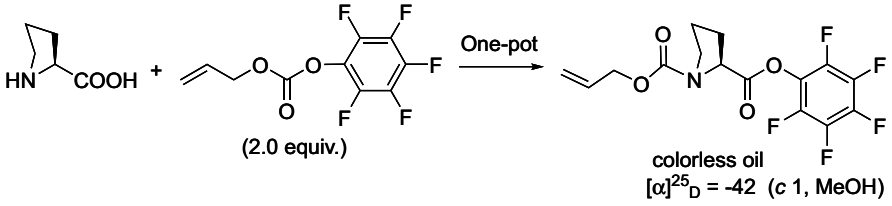
Methionine Sulfoxide [229]



mp. 253 °C;
$[\alpha]^{22}_D$ = +41.5 (*c* 2, 1N HCl)

A suspension of Met (3.0 g, 20.0 mmol) in H_2O (10 ml) was maintained at room temperature with stirring. A solution of H_2O_2 (30%, 2.2 ml) was added in small portions over a period of 30 min. A clear solution was formed. After a further hour at room temperature, absolute EtOH (100 ml) was added. Two hours later, the crystals were collected and washed with 95% EtOH (100 ml). Yield: 97%.

1.7.1.17 General Procedure for Concomitant Protection and Activation of Amino Acids Using Pentafluorophenyl Carbonate [139]



colorless oil
$[\alpha]^{25}_D$ = -42 (*c* 1, MeOH)

To a suspension of Pro (0.126 g, 1.1 mmol) and Aloc-OPfp (0.563 g, 2.0 mmol) in DMF (2 ml) at 0 °C was added pyridine (0.177 ml, 2.2 mmol) dropwise, and the mixture was allowed to attain 28 °C. The stirring was continued until the disappearance of the starting material. The mixture was then diluted with DCM (30 ml), washed with saturated citric acid solution (10 ml), H_2O (10 ml × 2) and brine (10 ml), dried over Na_2SO_4, and concentrated. The resulting product was purified by column chromatography (silica gel, 100–200 mesh) eluting with 5–15% EtOAc/hexane. Yield: 84%.

Note: The one-pot synthesis of Fmoc-amino acid pentafluorophenyl esters has been reported earlier. In this method, amino acid (1.1 equiv.) is treated with Fmoc-OPfp (1.0 equiv.) in a mixture of aqueous Na_2CO_3 and acetone at room temperature and after completion of the reaction (TLC), the mixture was acidified to pH 3 with concentrated HCl and extracted with EtOAc. To the organic layer, DCC (1.1 equiv.) was added at 0 °C and the mixture was stirred for 2 h. The DCU formed was filtered off and the filtrate was concentrated to afford Fmoc-amino acid pentafluorophenyl esters [138].

1.7.2 Deprotection Reactions

1.7.2.1 Removal of the Phth Group by Hydrazinolysis [284]

Phth-Gly–Gly-OH to H-Gly–Gly-OH The starting peptide (2.62 g, 10.0 mmol) was added to a 1 M solution of hydrazine hydrate in absolute EtOH (10 ml). The mixture was diluted with EtOH (30 ml) and refluxed for 1 h. The alcohol was evaporated and the residue was treated with 2 N HCl (25 ml) at 50 °C for 10 min, and then kept at room temperature for 30 min. The insoluble phthalylhydrazine was filtered off and the filtrate was evaporated. The residue was recrystallized from boiling EtOH to afford the purified hydrochloride salt (monohydrate, 1.73 g) that was then dissolved in H_2O (20 ml) and treated with the anion-exchange resin Amberlite IR4B until a drop of the solution gave no positive reaction for chloride ion with $AgNO_3/HNO_3$. The resin was filtered off, washed with H_2O, and the filtrate was concentrated to about 20 ml. The solution was heated on a steam bath, diluted with absolute EtOH until crystallization starts, and then allowed to cool to room temperature. The crystals were collected, washed with 95% EtOH, and dried. Yield: 81%.

1.7.2.2 Removal of the Nps Group

Nps-Lys(Boc)–Leu–Phe–Lys(Boc)–Lys(Boc)-OH to H-Lys(Boc)–Leu–Phe–Lys(Boc)–Lys (Boc)-OH [285] To a solution of ammonium rhodanide (0.16 g, 2.0 mmol) and 2-methyl-indole (0.26 g, 2.1 mmol) in MeOH (6 ml) and AcOH (18 ml), the starting peptide (1.12 g, 1.0 mmol) was added and allowed to stand at room temperature for 3 h. The solvent was evaporated, the residue was washed with warm (about 50 °C)

distilled H_2O (10 ml × 2), with 2 N NH_4OH (10 ml × 2), triturated with ether, and transferred to a filter. Trituration and washing with ether were continued until a white product was obtained that was dried *in vacuo* over P_2O_5 and KOH pellets. Yield: 90%; melting point 217 °C dec.; $[\alpha]_D^{20} = -4.2$ (*c* 1.2, AcOH).

1.7.2.3 Removal of the Z Group

1.7.2.3.1 Protocol A: Employing CH [45]

Z-Pro–Leu–Gly-NH$_2$ to H-Pro–Leu–Gly-NH$_2$ H_2 gas was bubbled through a solution of the starting peptide (3.2 g, 7.6 mmol) in EtOH (30 ml) containing aqueous HCl (1 equiv.) in presence of 5% Pd/C until completion of the reaction (TLC). The catalyst was filtered off, the filtrate was concentrated, and the residue was treated with a slight excess of NH_3 in $CHCl_3$. After NH_4Cl was filtered off, the filtrate was concentrated and the residue was recrystallized from H_2O. Yield: 80%.

Removal of Z through CH from Met-Containing Peptides [286]

Z-Trp–Met–Asp(OtBu)–Phe-NH2 to H-Trp–Met–Asp(OtBu)–Phe-NH$_2$ [46b] A solution of the starting peptide (7.87 g, 10.0 mmol) was dissolved in DMF (450 ml) followed by the addition of distilled H_2O (110 ml) and DIPEA (16 ml), and then 10% Pd-on-$BaSO_4$ (0.80 g). The mixture was stirred in an atmosphere of H_2 until no more gas was absorbed. The catalyst was filtered off and the filtrate was concentrated at 30 °C.

Note:

- Completion of the deprotection is determined by diluting the residue with DMF to about 175 ml followed by the addition of *p*-nitrophenyl ester of Z-Gly (3.64 g, 11.0 mmol). A day later, a spot test with ninhydrin (development of no purple color) indicates complete acylation of the amino group that was set free in the process of catalytic hydrogenation.
- The method cannot be applied when the peptides contain *S*-benzyl-Cys. Alternatively, Z group-protected peptides containing *S*-alkyl-Cys can be removed through catalytic hydrogenation in liquid ammonia solvent particularly in the presence of dimethylacetamide and TEA [46a].

1.7.2.3.2 Protocol B: Employing Silylhydride

Z-Tyr-OMe to Tyr-OMe [287] Neat triethylsilane (39.90 ml, 250.0 mmol) was added dropwise under an argon atmosphere to a stirred solution of Z-Tyr-OMe (8.2 g, 25.0 mmol) and Pd/C (1.23 g, 20% by weight) in 10: 1 MeOH/$CHCl_3$ (75 ml), and the mixture was stirred for 10 min. Upon completion of the reaction (TLC), the mixture was filtered through celite, the solvent was evaporated, and the product was purified on a short silica gel column. The yield was found to be quantitative.

Note: The same procedure has been used to cleave the Aloc group from Aloc-Leu-OMe in 5 min in quantitative yield [287].

1.7.2.3.3 Protocol C: Through CTH using 1,4-Cyclohexadiene as Hydrogen Donor [48b]

Z-Lys(Boc)-Thr(tBu)-OMe to H-Lys(Boc)-Thr(tBu)-OMe A solution of the starting peptide (5.52 g, 10 mmol) in absolute EtOH (50 ml) was maintained at 25 °C. A slow stream of N_2 was led above the surface of the solution and 10% Pd-on-charcoal (5.5 g) was added and the mixture was stirred, followed by the addition of 1,4-cyclohexadiene (8.0 g = 9.4 ml, 100.0 mmol). Upon completion of the reaction (TLC), the catalyst was filtered off and the filtrate was evaporated to leave a residue which was dried *in vacuo*. Yield: 98%.

Note:

- CTH is faster in glacial AcOH than in EtOH or in MeOH. In the case of the latter solvent, the rate is further reduced. With the former solvent, Met-containing peptides can also be used as substrates.
- For the removal of the $N^{\omega\prime}$-NO_2 group of Arg and N^{im}-benzyl group of His, more active Pd-black is used as the catalyst.
- CTH can also be carried out using cyclohexene, hydrazine, ammonium formate and 98–100% formic acid as hydrogen donors. With the last reagent as the hydrogen source in CTH, the Boc group as well as benzyl ethers have been removed concomitantly [48a].

1.7.2.4 Cleavage of the Fmoc Group

1.7.2.4.1 Method A: Using TAEA [67]

Fmoc-Gly–Gly–Phe–Leu-OBn to H-Gly–Gly–Phe–Leu-OBn Tris(2-aminoethyl)amine (7.5 ml, 50 equiv.) was added to a DCM solution containing the starting peptide and stirred for 30 min. During this time a white precipitate separated which dissolved readily in saturated NaCl solution. The reaction mixture was extracted with brine (10 ml × 3) and thrice (15 ml each time) with phosphate buffer (prepared by dissolving 90 g of $NaH_2PO_4 \cdot H_2O$ and 32.7 g of Na_2HPO_4 in 500 ml of H_2O, pH 5.5). There was no interference by either an emulsion or a precipitate. Additional DCM was used for back extraction. The clear organic layer was concentrated. The product can be directly used for coupling.

1.7.2.4.2 Method B: Using DEA: Simultaneous Removal of the Fmoc Group and 9-Fluorenylmethyl Ester [288]

Fmoc-Leu–Phe-OFm to H-Leu–Phe-OH The starting peptide (0.68 g, 1.0 mmol) was dissolved in DMF (9 ml) and DEA (1 ml), and the solution was allowed to stand at room temperature for 2 h. DEA and the solvent were evaporated at a temperature below 30 °C. The residue was triturated with a mixture of Et_2O (3 ml) and hexane (12 ml), and the solid product was collected and washed with a mixture of ether (5 ml) and hexane (5 ml). Yield: 94%; melting point 235–255 °C dec.; $[\alpha]_D^{23} = +37$ (*c* 1.4, AcOH); R_f 0.49 (*n*-butanol/AcOH/H_2O (4: 1: 1)).

1.7.2.5 Cleavage of the Boc Group

1.7.2.5.1 Protocol A: Removal of the Boc group with TFA in the Presence of Scavengers [103]

Boc-Leu–Leu–Gln–Gly–Leu–Val-NH_2 to H-Leu–Leu–Gln–Gly–Leu–Val-NH_2·TFA To a suspension of the starting peptide (0.37 g, 0.0.50 mmol) in 1: 1 PhOH/*p*-cresol (3 ml), TFA (2 ml) was added and the resulting solution was kept at room temperature for 1 h. TFA was evaporated and Et_2O (25 ml) was added to result in a precipitate. The latter was collected by centrifugation, washed with Et_2O (20 ml $\times$ 8), and dried in air under reduced pressure over P_4O_{10} to obtain the TFA salt of the peptide.

1.7.2.5.2 Protocol B: Cleavage of Boc Group with TMS/Phenol [105]

Boc-peptide resin was treated with 15 ml (per 1 g resin) of the 1 M Me_3SiCl/3 M phenol/DCM reagent for 5 min and then with a second 15 ml portion for 15 min. The washing protocol was as follows. The filtered resin was washed with DCM (15 ml), shaken for 3 min with 4% H_2O/DMF (15 ml), filtered, shaken for 3 min with 10% DIPEA/DMF, filtered, and washed 3 $\times$ 1 min with DCM (15 ml $\times$ 3). The resulting hydrochloride of the peptide-resin was neutralized with 10% DIPEA/DMF and washed with DCM in preparation for the next coupling cycle.

1.7.2.6 Transprotection of N^{α}-Protecting Groups: Fmoc-Met-OH to Boc-Met-OH [121e]

To a stirred solution of Fmoc-Met-OH (0.14 mmol) in DMF (1.2 ml) was added, under N_2 atmosphere, KF (0.98 mmol) followed by Et_3N (0.30 mmol). The solution was kept at room temperature until completion and Boc_2O (0.18 mmol) was then added. After stirring for several hours, the reaction was diluted with EtOAc, and washed with H_2O and cold 5% HCl, 5% $NaHC0_3$, and brine. The organic layer was dried (Na_2SO_4), filtered, and concentrated. The resulting crude product was purified by column chromatography to afford the product.

1.7.2.7 Selective Methyl Ester Hydrolysis in the Presence of the N^{α}-Fmoc Group

Fmoc-Gly–Phe–Pro-OMe to Fmoc-Gly–Phe–Pro-OH [145] The starting peptide was treated for 7 h with NaOH (1.2 equiv.) added to 0.8 M $CaC1_2$ in *i*PrOH/H_2O (7: 3, 10.6 ml) at room temperature. The hydrolyzate was neutralized with 1 M AcOH, evaporated, and the solid residue was dissolved in MeOH. Addition of H_2O resulted in a precipitate, which was filtered and extensively washed with H_2O. The crude peptide was recovered from the filter by dissolution into MeOH and evaporation of the solvent (purity greater than 80% by HPLC). The product was purified on silica gel (eluant: CH_2Cl_2/MeOH/AcOH, 5–10% MeOH, 1% AcOH); residual AcOH was eliminated by dissolution of the product in MeOH followed by precipitation and washing with H_2O. Yield: 85%. The product displayed high purity by HPLC and gave the expected fast atom bombardment mass spectrometry spectrum.

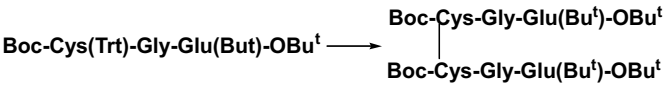
1.7.2.8 Cleavage of tert-Butyl Ester Using $BF_3 \cdot Et_2O$ [289]

Z-Cys–Gly–Gly–Gly–Cys(Bh)–Gly-OtBu to Z-Cys–Gly–Gly–Gly–Cys(Bh)–Gly-OH To a solution of the starting peptide (8.09 g, 0.01 mol) in AcOH (100 ml) was added ethyl mercaptan (5 ml, 0.0675 mol) and $BF_3 \cdot Et_2O$ complex (15 ml, 0.118 mol). The flask was swept with dry N_2 and kept at room temperature for 15 min. The product was precipitated by the addition of excess ether and reprecipitated from DMF. Yield: 95%.

1.7.2.9 Selective Cleavage of Phenacyl Ester in the Presence of the N^{α}-Nosyl Group [290]

Sodium benzenethiolate (5 mmol) was added cautiously to a stirred solution of N^{α}-Me-N^{α}-nosyl amino acid phenacyl ester (1 mmol) in DMF and the mixture was stirred at room temperature for 30 min under an inert atmosphere (N_2) with frequent monitoring (TLC, Et_2O/petroleum ether 70: 30 (v/v)). The solvent was evaporated and the residue was treated with 1 N NaOH and extracted with $CHCl_3$ (3–10 ml). The aqueous solution was acidified with 1 N HCl and then extracted with $CHCl_3$ (3–10 ml). The combined organic extracts were washed with brine, dried over Na_2SO_4, and evaporated to dryness to afford the product. Yield: oil, 70-87%.

1.7.2.10 Removal of the Trt Group (Iodolysis) [291]



The starting peptide (1.53 g, 3.0 mmol) and iodine (508 mg, 2.0 mmol) were dissolved in MeOH (25 ml), and stirred at room temperature for 1 h. The mixture was cooled in an ice-H_2O bath and decolorized by the dropwise addition of 1 M sodium thiosulfate in H_2O, diluted with H_2O (50 ml), and the precipitated material was filtered and dried. It was extracted with petroleum ether (10 ml × 3). The remaining solid was crystallized from EtOAc/hexane. Yield: 91%; melting point 150–152 °C.

1.7.2.11 Deprotection of the Pbf Group from Z-Arg(Pbf)-OH [19]

A sample of Z-Arg(Pbf)-OH (16 mg) was treated with TFA/H_2O (80: 20) at 37 °C. At regular intervals aliquots were diluted 4-fold with H_2O and analyzed by HPLC (mobile phase: H_2O/CH_3CN (A/B) containing 0.1% TFA. gradient: 13% B to 50% B, 15 min; 50% B, 13 min; flow rate: 3 ml min^{-1}). The average of two runs was used for comparison. A preparative run led to the isolation of product in 89% yield.

1.7.2.12 Removal of the Phenoc Group through Photolysis [253]

A solution of Phenoc-Thr-OH (3 mmol) in EtOH (100 ml) was irradiated in an atmosphere of argon at 0 °C in a Raynonet RPR 208 photochemical reactor with a 300-nm lamp After 4 h, a small amount of the starting material remained (TLC). After 14 h, the solvent was evaporated and the resulting solid residue was triturated with Et_2O. Yield: 75%.

1.7.2.13 Conversion of the DCHA Salt of N^{α}-Protected Amino Acids into Free Acids [292]

Nps-Thr(tBu)-OH·DCHA to Nps-Thr(tBu)-OH Finely powdered Nps-Thr(*t*Bu)-OH·DCHA (5.1 g, 10.0 mmol) was added to a two-phase system of EtOAc (40 ml) and a solution of $KHSO_4$ (2 g, 15.0 mmol) in H_2O (40 ml), and shaken by hand until the DCHA salt was completely dissolved. The aqueous layer was extracted with EtOAc (10 ml × 2) and the combined organic layers were washed with H_2O until they were free of sulfate ions, dried over Na_2SO_4, evaporated to dryness *in vacuo*, and the residue was dried over P_2O_5 for several hours.

Note: In the case of highly acid-sensitive protection, the free acid was converted into an active ester without isolation through carbodiimide-mediated esterification to prevent autocatalytic removal of the protection due to the acidity of the free carboxylic acid group.

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