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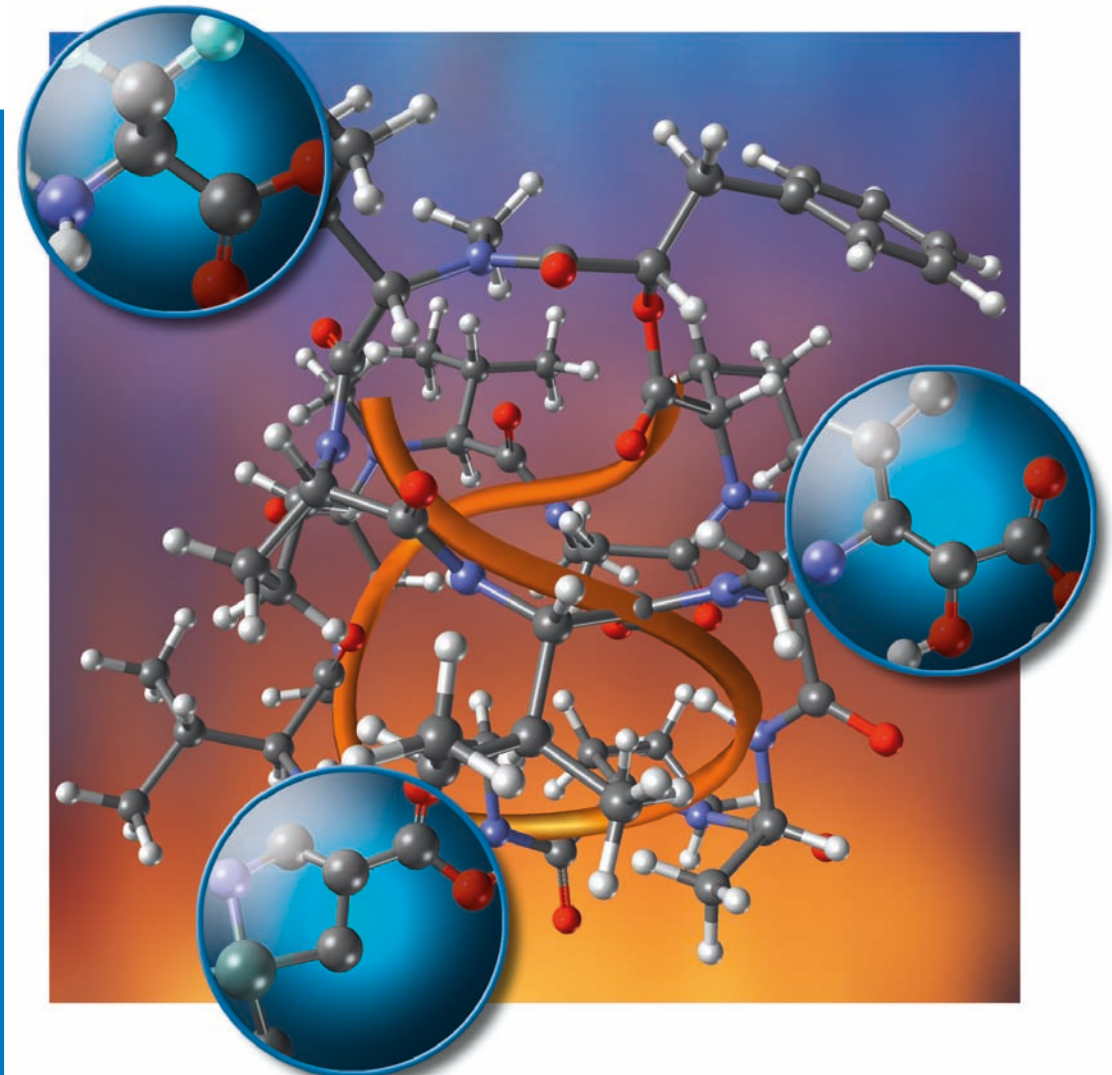
Amino Acids, Peptides and Proteins in Organic Chemistry

Volume 4
Protection Reactions, Medicinal Chemistry, Combinatorial Synthesis



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1
Protection Reactions

Vommina V. Sureshbabu and Narasimhamurthy Narendra

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

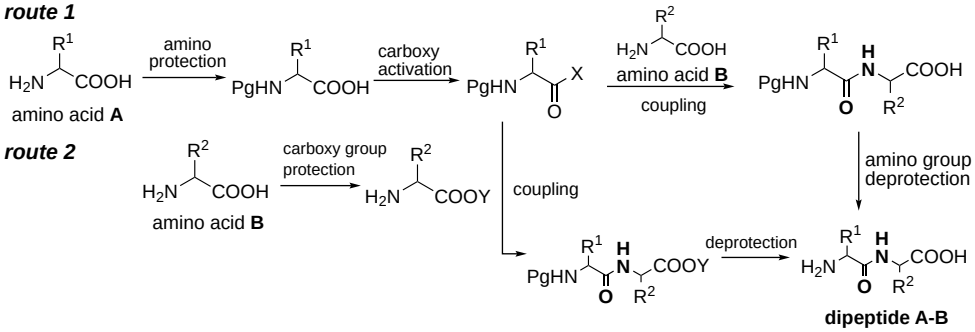

Figure 1.1 Illustration of synthesis of a dipeptide using α-amino and carboxy protections.

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.

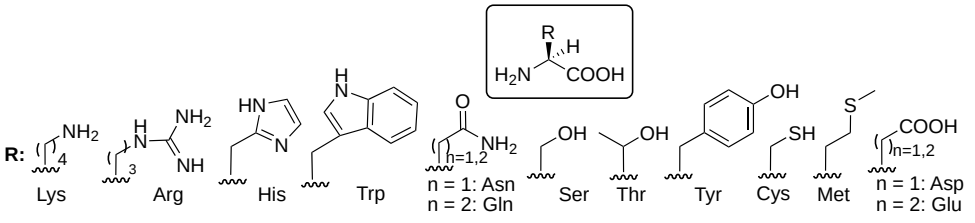

Figure 1.2 Side-chain functional groups of amino acids that entail protection.

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

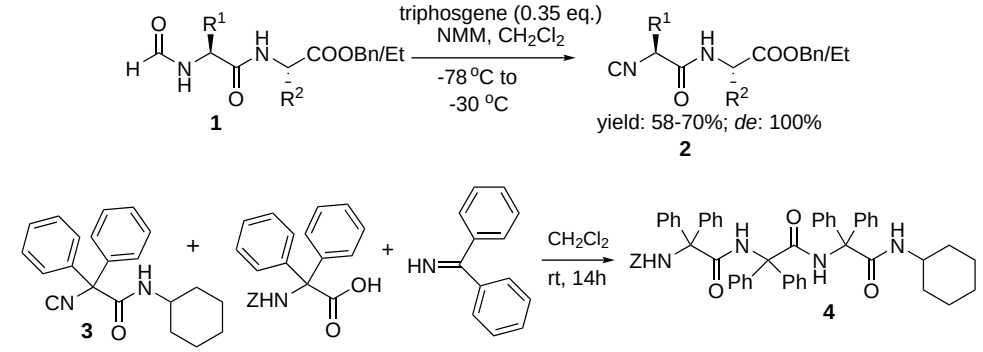

Figure 1.3 Synthesis of isocyanato peptides from *N*-For-protected peptide esters and the Ugi four-component reaction of α-isocyanato esters.

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

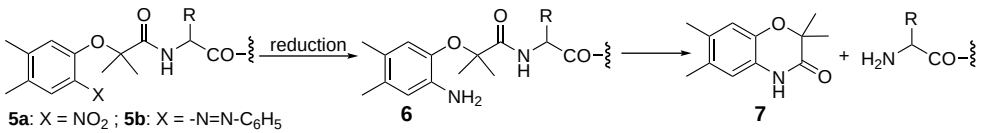

Figure 1.4 Cleavage of N^{α} protection via lactam formation.

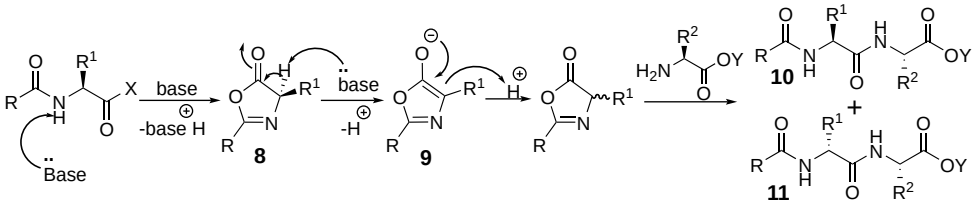

Figure 1.5 Racemization of N^{α}-acyl-α-amino acid derivatives.

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

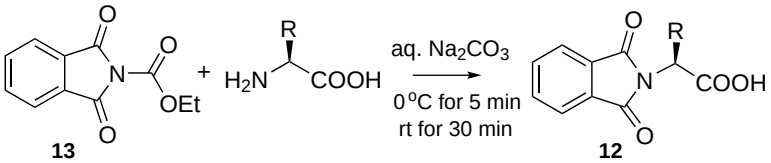

Figure 1.6 Preparation of N^{α}-Phth-amino acids.

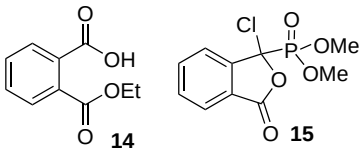

Figure 1.7 Phthaloylating reagents.

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

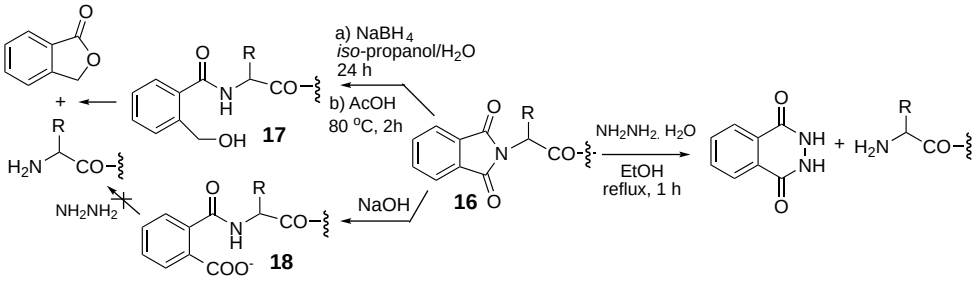

Figure 1.8 Cleavage of the N^{α}-Phth group.