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A New and Simplified Method for Hydrogenolytic Deprotection in Solution-Phase Peptide Synthesis

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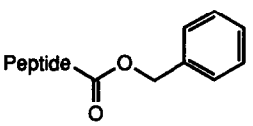
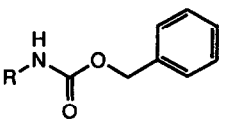
Abstract: An improved method for the deprotection of synthetic peptides by catalytic hydrogenation is described. The new method allows for precise control of counterion stoichiometry and affords the peptides in high purity and yield, while avoiding the problems usually associated with conventional deprotection methods.

Many dramatic advances have occurred in recent years in the field of solid-phase peptide synthesis. However, solution-phase methods have retained an important role in present-day peptide chemistry, despite the paucity of new information appearing in this area during the same period. In our laboratory, we require small oligopeptides, roughly six amino acids or fewer, in multigram quantities. Accordingly, solution-phase techniques remain the simplest and most expeditious for our purposes.

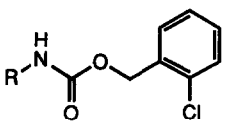
In a typical solution-phase peptide synthesis,[1] the product is first obtained in a blocked form, in which the terminal amino group, labile side-chain functional groups, and the terminal carboxyl group are blocked with groups removable by catalytic hydrogenation (see Figure 1). Such blocking groups are usually benzyl esters (of



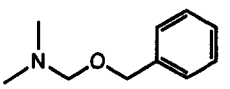
Benzyl Group



Benzyloxycarbonyl (CBZ) Group



2-Chlorobenzyloxycarbonyl (2-Chloro-Z) Group



Benzyloxymethyl (BOM) Group

Figure I. Some benzylic protecting groups used in peptide synthesis.

the C-terminus), benzyl ethers (e.g. hydroxyl side-chains of serine and threonine), or benzyl carbamates (for the N-terminus and amine side-chains of as lysine or arginine). Numerous variations on this concept have been

devised for specific functional groups or for certain reaction conditions. Several of these are shown in Figure 1. All take advantage of the lability of the benzylic functionality under the conditions of catalytic hydrogenation. In all of these cases, deprotection affords the free peptides, usually as salts, and the volatile by-products toluene and carbon dioxide, which are easily removed from the reaction mixture.

The chief disadvantages of conventional deprotection methods center on the harsh conditions often employed in these reactions, which usually involve dissolution of the blocked peptide in acetic acid or acetic acid/methanol[2] for periods often exceeding 24h. Such acidic conditions are known to facilitate the action of noble metal catalysts,[3] but they can also cause degradation or epimerization of peptides, particularly those containing labile residues such as tryptophan,[4] or histidine. Storage of peptide-acetate salts in solid form[5] has also resulted in degradation of the peptides.

In addition, peptides obtained as acetate salts are often difficult to characterize due to the presence of nonstoichiometric and highly variable amounts of acetic acid in the final (lyophilized) product. Thus, the effective molecular weight of the product can vary from one preparation to the next. Accordingly, we sought a means by which we could avoid these difficulties in our syntheses of small peptides.

Table I. Chloride Content of Selected Oligopeptides[6]

Peptide Sequence	Chlorine Calcd	Chlorine Found	Yield(%)
Gly-His-Glu·2 HCl	17.12	17.95	94
Gly-His-Leu·2 HCl	17.58	17.71	87
Phe-His-Phe·2 HCl	13.57	12.90	91
Gly-Arg-Lys·2 HCl	16.40	16.92	98
Gly-Arg-Lys·3 HCl	22.69	22.00	99
Gly-Ser-His-Lys·3 HCl	19.81	20.10	99
Pro-His-Lys-Phe·2 HCl	11.81	11.68	99
Val-Trp-Phe·HCl	7.28	7.76	79

We found that a highly active palladium catalyst, generated *in situ* from palladium (II) chloride under typical hydrogenation conditions (H_2, 60psig) in non-acidic solvents served to effect the desired conversion rapidly, and in high yield without degradation. Our method, based on a technique often employed in carbohydrate

chemistry,[7] furnishes free peptides as their hydrochloride salts with reproducible chloride ion stoichiometries in a controllable fashion. For each of the peptides shown in Table I, chloride content was within 1% of the theoretically calculated value.

Typical Procedure. A solution (or suspension) of the blocked peptide (1-20g) in 95% ethanol[8] (50-200mL, as needed) is diluted with water (up to a total volume of about 400mL) until an impenetrable milkiness or turbidity develops. This mixture is shaken on a Parr apparatus with palladium chloride ($PdCl_2$, 1 molar equivalent for dihydrochloride, 1.5 for trihydrochloride, etc.) under an atmosphere of hydrogen (5 atm or about 60psig) until the turbidity clears (usually about 30min.). The fine particles of palladium metal will then be clearly visible. This mixture is filtered to remove the catalyst and concentrated *in vacuo* to remove volatile organics (ethanol and the toluene produced in the reaction). The resulting aqueous solution is lyophilized. An aqueous solution of the solid obtained in this way is filtered through a 0.1μ or 0.2μ nylon membrane (such as the type used for filter sterilization) to remove any residual insoluble materials. Finally, this crystal-clear solution is lyophilized to give the product, typically as a fluffy white powder.

There are several important points which should be acknowledged in connection with this procedure. The first is the importance of choosing the correct stoichiometry for the reaction. The best results are obtained by allowing one equivalent of chloride for each **basic** nitrogen. Additional hydrochlorides associated with weakly basic sites are sometimes partially lost upon lyophilization. Weakly basic nitrogen atoms such as those of peptide bonds, and the side-chains of histidine and tryptophan need not be considered in calculating stoichiometry. Secondly, the method may be used successfully with peptides containing tryptophan without employing a side-chain protecting group for this reactive amino acid so long as oxygen is excluded during workup to prevent oxidation. Finally, large-scale procedures can be envisioned in which the reaction is performed using catalytic amounts of palladium chloride in aqueous ethanol in the presence of the required amount of hydrochloric acid.

In conclusion, it is important to recognize several notable advantages of this method. The most significant being its rapid reaction rate in comparison to the traditional methods described in the literature. The method also offers a visual endpoint, eliminating the need for chromatographic monitoring of the reaction. The chloride counterions usually exhibit the proper stoichiometry and are unlikely to undergo further reaction with the peptides. The mild conditions of the reaction give products of high purity in excellent yield. Thus, the debenzylation method we describe here affords a convenient route to multigram quantities of small peptides for research and development purposes.

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3. a. Rylander, P.M. **Catalytic Hydrogenation in Organic Synthesis**, Academic Press, Inc., San Diego, California, 1979, pp. 77, 195, 274.
   b. Martung, W.H.; Simonoff, R. *Org. React.* **1953**, *7*, 263.
4. Ref. 1, p. 2317, and references cited therein.
5. Procyte Corporation, unpublished data.
6. All products exhibited satisfactory ^{1}H NMR (500MHz), ^{13}C NMR (125MHz), CHN analysis, optical rotation, and chromatographic behavior (homogeneity by reverse-phase HPLC).
7. Ballou, C,E. *J. Am. Chem. Soc.* **1957**, *79*, 165. Ballou, C.E.; Roseman, S.; Link, K.P. *J. Am. Chem. Soc.* **1951**, *73*, 1140. This work, in turn, was based upon the method of Mozingo: Mozingo, R. *Org. Synth.* **1946**, *26*, 77.
8. We have investigated the use of solvents other than ethanol in this reaction, including methanol, isopropanol, dimethylformamide, and tetrahydrofuran with no discernable improvement in yield or reaction rate.

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