Schnabel E. Verbesserte Synthese von tert.-Butyloxycarbonyl-aminosäuren durch pH-Stat-Reaktion. (Improved synthesis of tert.-butyloxycarbonyl-amino acids by pH-stat reaction.) *Liebigs Ann. Chem.* **702**:188-96, 1967.
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The tert.-butyloxycarbonyl derivatives of free and partially protected amino acids are obtained in good yields by acylation with *t*-butylazidoformate at controlled pH. Sterically hindered amino acids also react smoothly. By variation of the pH values, the yield and the speed of the acylations can easily be determined for each amino acid derivative. [The *SCI*® indicates that this paper has been cited in over 540 publications since 1967.]

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"In 1957, the acid labile tert.-butyloxycarbonyl group (BOC) was introduced for N-protection of the amino groups of amino acids in peptide synthesis by McKay and Albertson[1] and independently by Anderson and McGregor.[2] After Schwyzer and his coworkers[3] had developed a generally feasible synthesis for BOC-amino acids using *t*-butylazidoformate[4] and magnesium oxide in water-dioxane as the solvent, these compounds found increasing application in peptide synthesis.

"When I started working for Bayer at Wuppertal in 1964, I was faced with the problem of how to synthesize analogues of the peptide hormone physalaemin. Our approach was to use extensively BOC-protected amino acids in conventional fragment condensation utilizing as many common intermediates as possible. Employing Schwyzer's method, most of the BOC-derivatives could readily be prepared but some were obtained in moderate yields only—especially BOC-Tyr(Bzl), BOC-Cys(Bzl), BOC-Ile, and BOC-Asp. Poor solubility and steric hindrance seemed to be the cause. Better yields of BOC-Tyr(Bzl) were indeed obtained with sodium hydroxide as base, but, during the reaction, Tyr(Bzl) precipitated. I next used an autotitrator to run the acylation at a constant pH of 10.4 in order to prevent precipitation. The reaction could easily be followed by the uptake of base and came out beautifully. BOC-Tyr(Bzl) was obtained in excellent yield and purity. On trying other amino acids whose acylation with *t*-butylazidoformate had been problematic, equally good results were obtained. Even the N-alkylamino acids reacted smoothly. It was amazing to watch the reaction of *t*-butylazidoformate with Pro. At pH 9.1, it proceeded with warming and was virtually complete within a few minutes. Even at pH 7.9, the acylation went to completion, though it took 12 hours. Next, my technician, J. Stoltefuss, suggested the use of buffers. He tried a few. The results looked promising but did not match those of autotitration.

"For several years, the autotitrator method was widely used for the preparation of the BOC-amino acids. Recently, di-tert.-butyldicarbonate[5,6] has become the reagent of choice for their synthesis, since it is commercially available, easy to handle, and an efficient acylating agent.

"I was really surprised at the acceptance of this paper: the use of *t*-butylazidoformate for the synthesis of BOC-amino acids was known,[3] autotitration was an already long known principle, too, and most of the derivatives had been synthesized previously. I, therefore, had hesitated for some time to publish this paper. Progress in peptide synthesis and especially Merrifield's solid phase method[7] produced a big demand for BOC-amino acid derivatives, of which only a few were commercially available at that time, and any procedure giving better yields was more than welcome. In addition, the paper contained a table with the physical constants of most BOC-amino acids and many colleagues have cited that data. In my opinion these are the reasons why this paper has become a *Citation Classic*."

1. **McKay F C & Albertson N F.** New amine-masking groups for peptide synthesis. *J. Amer. Chem. Soc.* **79**:4686-90, 1957.
2. **Anderson G W & McGregor A C.** *t*-Butyloxycarbonylamino acids and their use in peptide synthesis. *J. Amer. Chem. Soc.* **79**:6180-3, 1957. (Cited 310 times.)
3. **Schwyzer R, Sieber P & Kappeler H.** Zur Synthese von N-t-Butyloxycarbonyl-aminosäuren. *Helv. Chim. Acta* **42**:2622-4, 1959. (Cited 255 times.)
4. **Carpino L A.** Oxidative reactions of hydrazines. IV. Elimination of nitrogen from 1,1-disubstituted-2-arenesulfonhydrazides. *J. Amer. Chem. Soc.* **79**:4427-31, 1957.
5. **Pozdnev V F.** Application of di-tert-butyl pyrocarbonate for preparation of N-tert-butyloxycarbonyl derivatives of amino acids. *Khim. Prir. Soedin. SSSR* **1974**:764-7.
6. **Moroder L, Hallett A, Wünsch E, Keller O & Wersin G.** Di-tert.-butyldicarbonat—ein vorteilhaftes Reagenz zur Einführung der tert.-Butyloxycarbonyl-Schutzgruppe. *Hoppe-Seylers Z. Physiol. Chem.* **357**:1651-3, 1976.
7. **Merrifield R B.** Solid phase peptide synthesis. I. The synthesis of a tetrapeptide. *J. Amer. Chem. Soc.* **85**:2149-54, 1963. (Cited 1,340 times.)