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# This Week's Citation Classic

**Erlanger B F, Kokowsky N & Cohen W.** The preparation and properties of two new chromogenic substrates of trypsin. *Arch. Biochem. Biophys.* **95**:271-8, 1961. [Dept. Microbiology, Coll. Physicians and Surgeons, Columbia Univ., New York, NY]

**This paper describes the synthesis and properties of two new trypsin substrates, L-lysine p-nitroanilide and benzoyl-DL-arginine p-nitroanilide (BAPA). BAPA was found to be an excellent substrate, highly sensitive and specific and stable in the absence of enzyme. A novel method of synthesis was devised that was simple, economical, and which gave excellent yields. [The *SCI*® indicates that this paper has been cited over 775 times since 1961.]**

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"At the time of this research Bill Cohen was a postdoctoral fellow, fresh from F.F. Nord's laboratory.He is now a professor of biochemistry at Tulane. Nick Kokowsky had been a research assistant in my laboratory for several years and is now running his own chemical business. Bill was working on the catalytic mechanism of chymotrypsin by studying the reactivation of DEP-chymotrypsin by various nucleophiles. Our assay used acetyl-DL-phenylalanine B-naphthyl ester as the substrate, and included a color development with a diazonium salt.[1] The naphthyl ester was unstable, giving high blanks that differed in magnitude for each nucleophile.

"When we started studies on trypsin, we sought a substrate that would be stable and colorless and would yield a colored product upon tryptic hydrolysis.We prepared benzoyl-DL-arginine p-nitroanilide (BAPA) and L-lysine p-nitroanilide. BAPA was found to be an excellent substrate, highly sensitive, very specific and stable in the absence of trypsin. The product of tryptic hydrolysis, p-nitroaniline, is orange in color and can be measured directly, even in a colorimeter.[2]

"The synthesis we developed was esthetically satisfying in that (a) it was a one-step process followed by only one crystallization, (b) the yield was excellent, and (c) it was an original procedure that used $P_2O_5$ as a condensing agent (for which there was no precedent in the literature as far as I knew). The problem was to find a solvent and I happened to have a sample of diethylphosphite. It worked.

"We have used this assay as an easy way to teach students how to measure $k_{cat}$ and $K_m$ of enzyme-substrate reactions. We also prepared p-nitroanilide substrates for chymotrypsin,[3] and there now exist nitro-anilide substrates for many peptidases.

"The BAPA assay did not take hold for many years, a surprise to me since we found it more reproducible than titrimetric procedures with ester substrates. Its popularity grew suddenly when it was used in an indirect assay of trypsin inhibitor in the serum of patients with cystic fibrosis. Its ability to be automated was also probably a contributing factor. Although I cannot find the correspondence, it is my recollection that the paper was found not to be of general interest by the first journal to which we sent it.

"Although we cite both the Office of Naval Research (ONR) and National Institutes of Health (NIH) for support in the paper, the former was my major source of encouragement. It was through this agency that I was paid during my dissertation research and they gave me my first grant as an independent researcher. ONR, at that time helped many young researchers, thanks to people like Bill Consolazio and Orr E. Reynolds. Unfortunately, the Mansfield Amendment severely limited this kind of support on the part of ONR Coupled with the trend of other agencies toward contracts, program grants, and institutional grants, less money is now available to fund promising young scientists who can do a great deal with relatively small grants It is time that we correct this situation."

1 **Cohen W & Krlanger B F.** Studies on the reactivation of diethylphosphorylchymotrypsin. *J. Amer. Chem. Soc.* **82**:3928-34, 1960.
2. **Cohen W, Lache M & Erlanger B F.** The reactivation of diethylphosphoryltrypsin. *Biochemistry* **1**:686-93, 1962.
3. **Ealanger B F. Edel F, Edel & Cooper A G.** The action of chymotrypsin on two new chromogenic substrates. *Arch. Biochem. Biophys* **115**:206-10, 1966.