This paper reported the remarkable reactivity of phenylmethanesulfonyl fluoride (PMSF) toward chymotrypsin. PMSF is 10,000 times more reactive than the methane analog. We suggested that correct binding at the active site provides the driving force for the PMSF reaction. (The SC® indicates that this paper has been cited in over 485 publications since 1963.)

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August 2, 1983

"Landmark papers in the early 1950s established that certain enzymes react chemically with substrates to form covalently bonded enzyme-substrate intermediates. Thus, the stage was set for the design of 'active-site directed' irreversible inhibitors as probes for enzyme mechanisms in the 1960s.

"As a graduate student with David Nachmansohn at Columbia University, I was expected to work on acetylcholinesterase. But my interest turned to a paper on the inactivation of chymotrypsin by dansyl chloride. This sulfonyl chloride reacts with a histidine residue at the active site, instead of serine. Although evidence that the Nazi nerve gas DFP reacted at a serine residue seemed irrefutable to some, others argued for nucleophilic attack by a histidine residue, followed by transfer of the phosphoryl group to a nearby serine hydroxyl group. In contrast, model studies indicated that transfer of a sulfonyl group from histidine to serine was unlikely. Since sulfonyl fluorides are much less reactive than phosphoryl fluorides, I thought that a sulfonyl fluoride would react at the active site only if it were juxtaposed against the attacking nucleophile. If the R group were phenylmethyl instead of DFP's biologically irrelevant isopropyl group, the sulfonyl fluoride might mimic phenylalanine substrates and react with the correct nucleophilic group. Nachmansohn liked the idea and a research plan was sent to the National Science Foundation. But he did not let me start in the lab until I had filled two notebooks with handwritten abstracts—not Xerox copies—of papers on chymotrypsin. The work proceeded smoothly, due largely to the superb guidance of Allen Gold, then a post-doctoral fellow in Nachmansohn's group. When the time to submit the manuscript arrived, Nachmansohn felt Al and I should be the authors. First author was decided by a toss of a coin. The manuscript received two diametrically opposed reviews and was initially rejected by the editor. Nachmansohn demanded a third reviewer and the manuscript was accepted verbatim.

"Although cited often by researchers designing new enzyme inhibitors, citations also occur in papers on the isolation and purification of proteins from a wide variety of biological systems. Phenylmethanesulfonyl fluoride (PMSF) is added to buffers in place of the highly toxic DFP to block proteases. Unfortunately, PMSF doesn't always work: after all, it was designed to fool chymotrypsin.

"PMSF was a new compound. Twenty years later the field of designing new enzyme inactivators is still very active. Perhaps the most ingenious are the recent 'suicide substrates.'"