AMINO ACID AND PEPTIDE THIOESTERS: NEW SENSITIVE SUBSTRATES FOR BOVINE FACTORS IX, XI, XII, THROMBIN, PLASMA KALLIKREIN AND TRYPsin.

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A series of amino acid and peptide thioesters have been synthesized as sensitive assay substrates for the serine proteases involved in blood coagulation. Each substrate had a P_4 Arg residue. The thiol leaving group (P_4^*) and the P_4 amino acid residues were varied. The thioesters have much higher K_m values than the corresponding amides such as i-nitrophenylacetil- and i-amino-i-methylcoumarin amides. In addition, the thiol released upon enzymatic hydrolysis of the substrates, can be measured chromogenically by use of a thiol reagent such as 4,4' di-thiodipyridine contained in the assay mixture. Thioesters are among the most sensitive assay substrates for coagulation serine proteases due to the combination of a high turnover rate with the ease of detection of the hydrolysis product. In addition they can be utilized with enzymes such as factor IX for which no other suitable synthetic substrate is currently available.