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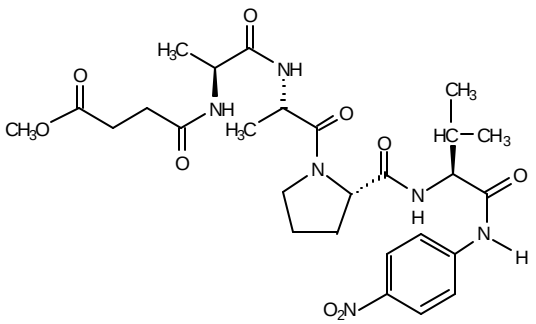
ProductInformation

N-METHOXYSUCCINYL-ALA-ALA-PRO-VAL P-NITROANILIDE
Product Number **M4765**
Storage Temperature -20°C

CAS #: 70967-90-7
Synonyms: MeO-Suc-Ala-Ala-Pro-Val-NA[1,2] MeO-Suc-Ala-Ala-Pro-Val-pNA[3], MeO-SucAAPVpNA[4]

Product Description



Appearance: powder[5]
Molecular Formula: $C_{27}H_{38}N_6O_9$
Molecular Weight: 590.6

Methods of synthesis have been reported.[2,6]

MeO-SucAAPVpNA is an excellent synthetic substrate for human leukocyte elastase (HLE). HLE is one of the most destructive proteases in the body and its role in development of pulmonary emphysema has been investigated.[3,12] This peptide is selectively hydrolyzed by HLE, but it is not a substrate for human leukocyte cathepsin G.[1,2] The mechanism[6,9,12] and kinetic constants[1-3,8] of the elastase reaction have been reported. Elastase from different species and cells will hydrolyze this substrate.[1-3,7,8] MeO-SucAPVVpNA is also cleaved by an elastase-like protease important for the synthesis of platelet activating factor (PAF) induced by tumor necrosis factor-α (TNF-α) and interleukin-1α (IL-1α).[10] MeO-SucAAPVpNA has been used to measure neutrophil elastase activity in bronchoalveolar lavage fluid of cystic fibrosis patients.[11]

The rate of enzymatic hydrolysis of the substrate is followed by the increase in absorbance due to the release of 4-nitroaniline. The absorbance is routinely measured at 400 nm or higher. The following molar extinction coefficients have been reported for the free 4-nitroaniline:

410 nm[13], $E^M(M^{-1}cm^{-1})$ = 8,800
405 nm[14], E^M = 9,500
400 nm[15], E^M = 12,300

The absorption maximum for the free 4-nitroaniline is at 380 nm (E^M = 13,500); however, at that wavelength there is an overlap with the absorption spectrum of the anilide substrate (maximum at 315 nm).[13]

Preparation Instructions

The methoxysuccinyl group increases solubility relative to acetyl substitution; however, MeO-SucAAPVpNA still has limited solubility in water (0.5 mg/ml).[5] The peptide may be prepared as a stock solution in dimethyl sulfoxide (DMSO) at 15 mM prior to dilution into aqueous assay systems.[7] It is also soluble in other organic solvents: N, N-dimethylformamide – DMF (25 mg/ml)[5], methanol (1 mg/ml)[5] and 1-methyl-2-pyrrolidinone (20 mM).[16] DMSO has been preferred over DMF for use to prepare stock solutions[7] and the substrate has been reported to breakdown rapidly in 1-methyl-2-pyrrolidinone.[17] Solvent effects on HLE results should be considered as DMSO has been reported to stimulate HLE activity.[1] Substrate solutions have also been prepared in 20 mM Tris-HCl, pH 7.5, with the addition of human serum albumin (10 µg/ml).[3] The peptidyl linkage of the p-nitroaniline moiety is relatively stable to autohydrolysis. No interference was observed due to spontaneous hydrolysis in assays performed at room temperature for 27 hours[1] and 72 hours.[18]

References

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