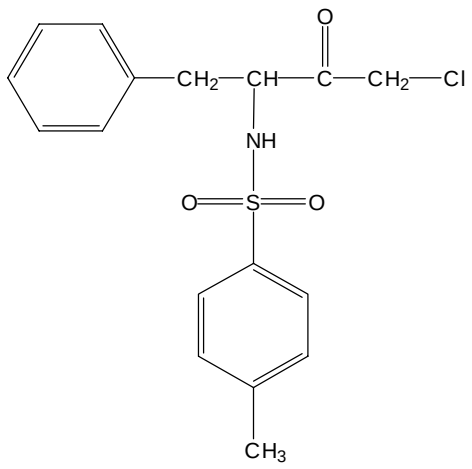
ProductInformation

N-TOSYL-L-PHENYLALANINE CHLOROMETHYL KETONE
Sigma Prod. No. T4376

CAS Number: 402-71-1
SYNONYMS: TPCK; Tosylphenylalanylchloromethane; L-Tolylsulfonylphenylalanyl Chloromethyl Ketone; N-Tosyl-L-Phenylalaninechloromethane;[1] L-1-Tosylamido-2-Phenylethyl Chloromethyl Ketone; L-N-(α-(Chloroacetyl)Phenethyl)-p-Toluenesulfonamide[2]; Tos-Phe-CH_2Cl[3]



PHYSICAL DESCRIPTION:

Appearance: white powder[4]
Melting Point: 106-108°C[2]
Molecular Formula: $C_{17}H_{18}ClNO_3S$
Molecular Weight: 351.8

METHOD OF PREPARATION:

TPCK is synthetically prepared.[5] A method of preparation has been reported.[6]

STABILITY / STORAGE AS SUPPLIED:

TPCK is expected to be stable for at least two years when stored desiccated at -20°C.[4]

SOLUBILITY / SOLUTION STABILITY:

Stock solutions of 10 mM can be prepared in methanol or ethanol and are stable for several months at 4°C.[3] TPCK is soluble in DMSO[7]; preparation of 10 mg/ml solution was described.[8] The effective concentrations in aqueous solutions are in the range of approximately 10-100 μM. Working solutions are stable only for several hours.[3]

USAGE / APPLICATIONS:

TPCK irreversibly inhibits the serine protease α-chymotrypsin.[6,9] TPCK has been shown to specifically alkylate the histidine-57 moiety in the active center of chymotrypsin and chymotrypsin-like serine proteases.[6,10,11] The nature of the enzyme-inhibitor complex and the mechanism of inactivation have been reported.[12,13,14]

USAGE / APPLICATIONS: (continued)

TPCK inhibits the active enzyme and not the zymogen precursor nor enzyme-inhibitor complex.[6,11] Trypsin (not inactivated) can be treated with TPCK for removal of chymotrypsin activity.[15] TPCK also inactivates some cysteine proteases[9] such as bromelain[16,17], ficin[18] and papain[19,20] by reacting with the active sulfhydryl group of the enzyme rather than on the imidazole group of a histidyl residue, as in the case of chymotrypsin. TPCK inhibited the catalytic subunit of cAMP-dependent protein kinase in both rat and rabbit muscle[21,22] and protein kinase C (IC_{50}=8mM)[23] probably by the alkylation of a sulfhydryl-containing amino acid residue in the enzyme active center. TPCK (IC_{50}, 5 μM) inhibited the mitogen-induced activation of $pp70^{s6k}$, a mitogen-regulated serine/threonine kinase involved in the G^1 to S phase transition of the cell cycle.[24]

TPCK (25 μM) reportedly induced early morphological and biochemical changes associated with apoptosis, inhibited internucleosomal cleavage in rat thymocytes, and (at 10 μM) in human promyelocytic leukemic cell line (HL-60) and affected other apoptotic events induced by campothecin (CAM).[7,25] TPCK (30 μM) induced tyrosine phosphorylation of a substrate (molecular weight, 42,000) in HL-60 cells and in human monocytes in conjunction with inhibition of apoptosis-associated CAM-induced internucleosomal DNA fragmentation. The reported results suggest a link between protein phosphorylation (as a signalling event) and regulation of apoptosis.[26] TPCK reacted with the Rb-binding core of human papillomavirus HPV-18 E7 oncoprotein and destroyed its Rb-binding ability.[27] TPCK (5×10^{-4}M) was shown to be a selective irreversible inhibitor of the complex of S_1S_3-factors in the cell-free protein-synthesizing system from B. stearothermophilus.[28] TPCK (25 μM) completely inhibited induction of NF-kB (transcriptional activator protein) activity as well as the decay of the subunit IkB (necessary for activation) in response to phorbol 12-myristate 13-acetate (PMA) in cells.[29] TPCK (mM concentration) inhibited E. coli proteases, Re (1 mM); Fa (80% at 0.5 mM) and So (1 mM).[30] TPCK weakly inhibited chymase (I_{50}=240 μM), a serine protease, in rat peritoneal mast cells.[31]

In vitro nitric oxide production from immunostimulated alveolar macrophages of the mice and rat was inhibited by TPCK ($3x10^{-7}$-$3x10^{-4}$M).[32] TPCK interfered with the lipopolysaccharide induced nitric oxide synthase gene expression in rat alveolar macrophages.[33] TPCK (100-200 μg/ml) inhibited the growth of simian virus 40-transformed and untransformed 3T3 cells probably by inhibition of cellular protein synthesis.[34] Addition of 20-30 μg/ml of TPCK to HeLa cells, virus-transformed 3T3 mouse fibroblasts and mouse plasmacytoma culture cells irreversibly inhibited initiation of protein synthesis.[8] TPCK irreversibly inhibited the interaction between the Elongation Factor Tu and phenylalanyl transfer RNA and prevented its transfer to the ribosome for formation of polyphenylalanine.[35] TPCK (5.7 μM) increased arachidonic acid metabolism (prostacylin production increased) in rat liver cells stimulated by agonist agents.[36]

USAGE / APPLICATIONS: (continued)

TPCK (284 μM) inhibited the activity of purified CMP-sialic acid:lactosylceramide α(2→3) sialytransferase (GM_3 synthase, a GM_3 ganglioside-forming enzyme) from rat liver.[37] TPCK (1 mM) inhibited about 78% of a chymotrypsin-like activity of human leucocyte granules.[38] To prevent proteolytic degradation throughout isolation of proteins, 1 mM each of TPCK and trypsin inhibitor TLCK (L-tosyl-lysine chloromethyl ketone) was used in the isolation of histones from chicken erythrocytes.[39]

GENERAL NOTES:

TPCK is an irreversible inhibitor of chymotrypsin, of chymotrypsin-like serine proteases, and of some cysteine proteases.[3,16,18,40,41] TPCK which is hydrophobic and of relatively low molecular weight is likely to penetrate the plasma membrane and act within the cell, i.e., affecting cell apoptotic events.[7,25]

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