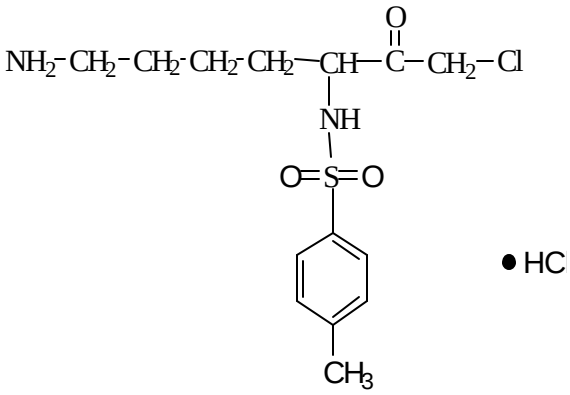
3050 Spruce Street
Saint Louis, Missouri 63103 USA
Telephone 800-325-5832 • (314) 771-5765
Fax (314) 286-7828
email: techserv@sial.com
sigma-aldrich.com

ProductInformation

N- a-TOSYL-L-LYSINE CHLOROMETHYL KETONE HYDROCHLORIDE
Sigma Prod. No. T7254



CAS NUMBER: 4272-74-6
SYNONYMS: TLCK; Tos-Lys-CH_2Cl[1]; L-1-Chloro-3-[4-tosylamido]-7-amino-2-heptanone HCl[1]; p-Toluenesulfonamide, N-[5-Amino-1-(Chloroacetyl)Pentyl]-Hydrochloride[2]

PHYSICAL PROPERITIES:

Appearance: White to pink powder[3]
Molecular Formula: $C_{14}H_{21}ClN_2O_3S.HCl$
Molecular Weight: 369.3

METHOD OF PREPARATION:

TLCK is synthetically prepared.[4] Methods for preparation have been described.[5,6]

STABILITY / STORAGE AS SUPPLIED:

TLCK is stable for at least two years when stored desiccated at -20EC.[3]

SOLUBILITY / SOLUTION STABILITY:

TLCK is soluble in water. Stock solutions of 10 mM can be prepared in 1 mM HCl, pH 3.0 or in buffer at pH # 6.0 (solutions are very unstable above pH 6.0 at 25EC, i.e. about 48% will decompose in about 5 minutes at pH 9.0).[1,5] Solutions should be prepared fresh. The effective concentrations are 10-100 µM.[1] TLCK has also been solubilized in methanol at 50 mg/ml[3] and in DMSO at a concentration of 5 mM.[7]

USAGE / APPLICATIONS:

TLCK is an irreversible inhibitor of the serine protease, trypsin (inactivates trypsin most rapidly at pH 7.5) and many trypsin-like serine proteases. The histidine-46 residue located in the active site of the enzyme is alkylated by TLCK.[5,6] TLCK does not inhibit trypsinogen nor complexes of trypsin-inhibitor such as trypsin-benzamidine.[5]

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USAGE / APPLICATIONS: (continued)

The nature of the enzyme-TLCK complex and mechanism of inactivation by peptide choromethyl ketones have been reported.[5,8,9] TLCK also inhibits plasmin, thrombin (10 mM TLCK to 100 units/ml of thrombin[10]) and the thiol enzymes, ficin, bromelain, clostripain and papain.[11-23] The kinetics of inhibition of papain were reported.[11] TLCK alkylates the essential sulfhydryl group involved in the catalytic mechanism for the thiol proteases rather than the imidazole group of particular histidyl residues (as in the case of trypsin and thrombin[23] for example). TLCK inhibits some mouse submaxillary gland proteases such as Endoproteinase Arg-C[1,24] and it inhibits Endoproteinase Lys-C (1 mM).[1,25] TLCK is reportedly a potent inhibitor of Protein Kinase C (IC_{50}=1 mM)[26] and it inhibited the catalytic subunit of the Cyclic AMP-dependent Protein Kinase in rabbit and rat muscle.[27,28] TLCK reacted with the Rb-binding core of human papillomavirus HPV-18 E7 oncoprotein and destroyed its Rb-binding ability.[29] In vitro nitric oxide production from immunostimulated alveolar macrophages of the mice and rat was inhibited by TLCK ($3x10^{-7}$-$3x10^{-4}$M).[30] A later study reported that TLCK interfered with the lipopolysaccharide induced nitric oxide synthase gene expression in rat alveolar macrophages.[31] TLCK (50 μM) prevented in situ DNA endonucleolytic cleavage during apoptosis of HL-60 cells and rat thymocytes induced by different agents.[7] TLCK inhibited apoptosis (most likely by inhibition of trypsin-like proteases) in thymocytes.[32] TLCK (IC_{50}=50 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, in different cell types.[33] TLCK (135 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.[34] TLCK reportedly inhibited protein breakdown in E. coli cells starved for a carbon source and macromolecular synthesis in E. coli.[35,36]

Tryptase, a serine trypsin-like protease in rat peritoneal mast cells, was inhibited by TLCK (I_{50}=230 μM).[37] TLCK also inhibited human complement C6 (4×10^{-7}M) in the range of $1O^{-2}$-$1O^{-3}$M[38], dog urinary kallikrein (K_i=6.2×10^{-3}M)[39], human enterokinase[40], and boar acrosin (at 2 μM).[41] Rat adenylate cyclase was inhibited by TLCK presumably by modification of a reactive thiol group.[42] TLCK (0.1 M) was reported to inhibit tumorigenesis in mouse skin.[43]

GENERAL NOTES:

TLCK has been used in the preparation of chymotrypsin free of contaminating trypsin activity (chymotrypsin is not inhibited).[1] To prevent proteolytic degradation throughout the isolation of proteins, 1 mM each of TLCK and TPCK (N-Tosyl-L-Phenylalanine Chloromethyl Ketone), a chymotrypsin inhibitor, was used in the isolation of histones from chicken erythrocytes.[44] TLCK 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.[7,32]

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