

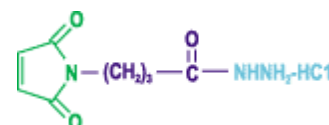
MPH, MCH(EMCH), KMUH, MPBH SH and CHO reactive Crosslinkers

Product Description

Catalog number: UPG9909A, 50mg

Name: MPH

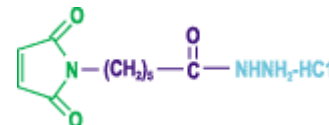
Formula : MaleimidoPropionic acid Hydrazide, HCl
 $C_8H_{12}N_3O_3Cl$, M.W.= 233.65



Catalog number: UPG9910A, 50mg

Name: MCH, EMCH

Formula : MaleimidoCaproic acid Hydrazide, HCl
 $C_{10}H_{16}N_3O_3Cl$, M.W.= 261.71



Catalog number: UPL7722A, 50mg

Name: KMUH

Formula : N-(k-Maleimidoundecanoic acid)hydrazide
 $C_{15}H_{25}N_3O_3$ M.W.= 295.4

UPL7722B, 100mg

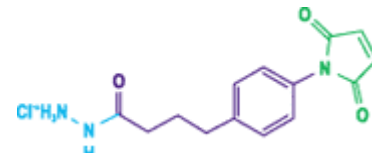


Catalog number: UP09835A, 100mg

Name: MPBH

Formula : 4-(4-N-MaleimidoPhenyl)butyric acid Hydrazide.HCl
 M.W.= 309.5

UP09835B, 50mg



Storage : +4°C (long term: -20°C) , protect from moisture and light. (L)

General Information

Cross-linkers are chemical reagents used to conjugate molecules together by a covalent bond. Several atoms separate the 2 molecules, forming the **spacer**. The conjugate associates the characteristics and biological activities of each component.

Cross-linkers have become important tools for the preparation of conjugates used in a lot of immunotechnologies, and for protein studies (structure, interactions, activity, degradation...). To that point, **heterobifunctional crosslinkers** are probably the most interesting, because they present 2 reactivities that allow the conjugation of molecules in a defined manner, avoiding notably the formation of dimers and polymeres. The choice of reactivities is determinant for the design of the right conjugate. An important other thing to consider is the nature and length of the spacer.

Uptima offers 3 sulfhydryl and aldehyde reactive cross-linkers of high quality to answer the needs of coupling biomolecules for biological and immunoassays like (other cross-linkers are available): MPH, MCH and KMUH differ by the length of the spacer, and are used classically to cross-link a thiolated protein to a carbohydrate, allowing well directed conjugation.

Applications involve notably glycosylated or reduced proteins, peptides, nucleic acids:

- Obtention of immunogen carrier-hapten
- Obtention of labeled affine probes: for example, antibodies coupled to enzyme for immunoblotting, fluorophore-peptides conjugates for the study of receptors, enzyme-drugs for using as tracers in ELISA...
- Obtention of oligomeric conjugates : conjugates of glycolipids for immunization, structural studies...
- Immobilization of ligands: grafting haptens onto cells, gels or functionalized supports...
- Obtention of biologically active conjugates: specific antibody coupled to drugs for immunotargeting techniques, immunotoxins, ...

This sheet describes cross-linkers that contains a reactivity toward **sulfhydryls**, through the maleimide group, and a reactivity toward **carbohydrates**, through the hydrazide group.

Scientific and Technical Information

- The **spacer arm** span 4 atoms (MPH),
6 atoms = 11.8 Å length (MCH),
11 atoms = 19.0 Å (KMUH).
It is linear but flexible, allowing the interaction of conjugated molecules on both sides.
MPBH spacer is 17.9 Å long, and contains a aromatic cycle that reduces slightly the flexibility. It is also more immunogenic.
- **MPBH solubility** is 620 mM in DMSO, 1500 mM in DMF and around 1 M in aqueous buffers.
- The **maleimide** group reacts very specifically with sulfhydryls –SH at neutral pH >6.5. The reaction is rapid (a few minutes for cystein), but in the absence of –SH, it is well stable. The hydrolysis forming maleimic acid becomes noticeable when pH go up 8.0, where the reactivity with amines begins to be possible. In usual conditions, pH6.5-7.5, one should start with a ratio of 10-20 moles of maleimide per mole of protein. With SH-peptides, a molar 1:1 incubation ratio allows usually almost 1:1 coupling.
Sulfhydryls are available in some proteins and peptides (often synthesized with a N-terminus Cysteine), or can be generated by reduction with DTT (#UP28425) or introduced with Iminothiolane (#UP42425) or SATA (#UP84235)
- The **hydrazide** group reacts specifically with **aldehyde**, forming a stable hydrazone bond.
 $R-CHO + N_2-NH-R' \rightarrow R-CH=N-NH-R'$
Aldehydes are present in reducing oses, or can be generated from cis-diol found notably in carbohydrates by specific oxidases such as galactose oxidase, or by mild oxidation with 10mM NaIO₄ at RT in the dark (Chamow 1978):
 $R-CH(OH)-CH(OH)-R + NaIO_4 \rightarrow R-CHO$
Rem: Hydrazide also reacts with **carboxylic acids** in the presence of EDAC (#UP520050)
 $R-COOH + Hydrazide-R' + EDAC \rightarrow R-CO-NH-NH-CO-(CH_2)_4-R'$
Rem: As hydrazone bond is not enough stable at very low pH, this can be converted to hydrazine by reaction with NaBH₄ for some applications (O'Shannessey 1990).
- The **reaction scheme** of the conjugation should be designed depending on each application. The ratios of cross-linker to molecules and reaction steps should be determined in each step for each application.

One could for example activated first the SH bearing molecule, because maleimide react quickly and very specifically at pH 6.5-7.5, then add the aldehyde bearing molecule. Desalting of by product may be performed by dialysis (CelluSep) or gelfiltration.

When a molecule bears both chemical groups, one should choose , in order to avoid the formation of dimeres, either to block undesired SH on molecule for the activation step, or of the excess of maleimide for the coupling step, or to invert the steps. At the opposite, EDTA can be added in buffers to prevent the reoxidation of SH into disulfides (Ishikawa 1983).

Specific protocol can be found in the literature (Chamow 1992): IgG can be oxidized for creating CHO groups that can be activated by the hydrazide group of the cross-linker (ratio 16:1, 2H at room temperature in acetate 0.1M pH5.5). After desalting, the maleimide that is relatively stable during the activation step, reacts with a SH-bearing protein like b-galactosidase or hemoglobin (ratio 1:1, 1H at room temperature in PBS pH7.0). The cross-linking of the antibody through their carbohydrate residue, located on the Fc portion, allows to maintain excellent immunological recognition (antigen binding).

Other Information

For use *in vitro* only, not for diagnostic.

Literature

Trail, P.A., *et al.* (1993). Cure of xenografted human carcinomas by BR96-doxorubicin immunoconjugates. *Science* **261**, 212-215.
Chamow, S.M., Kogan, T.P., Peers, D.H., Hastings, R.C., Byrn, R.A. and Askenaszi, A. (1992). Conjugation of soluble CD4 without loss of biological activity via a novel carbohydrate-directed cross-linking reagent. *J. Biol. Chem.* **267**(22), 15916-15922.
Chamow S.M. et al, *Biochem J.*, 1978, **173**, 723-

For any information, please contact Uptima, or your local distributor.

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