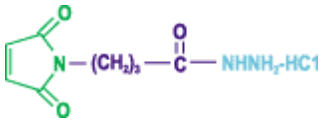
FT-G9910A

MPH, EMCH, KMUH, MPBH

SH and CHO reactive crosslinkers

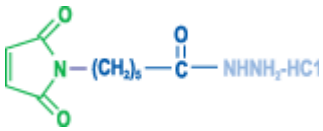
Products description

Catalog number: UPG9909A, 50mg
Name: **MPH (BMPH)**
Formula : MaleimidoPropionic acid Hydrazide, HCl salt
MW= 220.64
MPH, TFA salt, #L7725A, 100mg MW= 298



MW (salt free): 184.18 – Spacer 8.1A

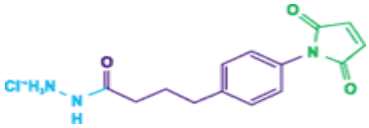
Catalog number: UPG9910A, 50mg
Name: **EMCH (MCH)**
Formula : MaleimidoCaproic acid Hydrazide, HCl salt
MW= 261.71
EMCH, TFA salt, #90038A, 50mg MW= 339.27



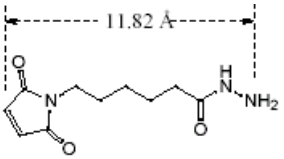
MW (salt free): 225.24

Catalog number: UPL7722A, 100mg UPL7722B, 50mg
Name: **KMUH**
Formula : N-(k-Maleimidoundecanoic acid)hydrazide, TFA salt
(TFA salt) MW= 409.41
(salt free) *MW= 295.4*

Catalog number: UP09835A, 100mg UP09835B, 50mg
Name: **MPBH**
Formula : 4-(4-N-MaleimidoPhenyl)butyric acid Hydrazide.HCl salt
MW= 309.5



Catalog number: BI0691, 100mg
Name: **MPS-EDA** TFA
Formula : NHS-3-maleimidopropionate TFA salt
Syn.: NHS-3-maleimidopropionate Succinimide ester; N-(ß-Maleimidopropyloxy) succinimide ester
MW= 325.24
A unique Sulfhydryl and Carbonyl reactive crosslinker, highly water soluble



Spacer 11.8A (10 atoms)

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 bound. Several atoms separate the 2 molecules, forming the '**spacer arm**'. The conjugate associates the characteristics and biological activities of each components.

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 polymers. 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 sulfhydryl and carbonyl 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 typically to cross-link a thiolated protein to a carbohydrate, allowing well directed conjugation.

This sheet describes cross-linkers that contains a reactivity toward **sulfhydryls**, through the maleimide group, and a reactivity toward **carbonyls (carbohydrates)**, through an hydrazide group or a ester (MPS-EDA)

Scientific and Technical Information

- The **spacer arm** spans 4 atoms (MPH), 6 atoms =11.8 Å length (MCH), or 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.
MPS-EDA spacer is 10 atoms / 10.7 Å length.

- 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 cysteine), 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 naturally on the biomolecule2 to conjugate, usually in cysteine residues of proteins, or introduced by chemistry as in peptides (often synthesized with a N-terminus Cysteine). Note that they can be oxidized (i.e. forming disulfide bridges), thus non-reactive for conjugation, and often involved in protein function. When free sulfhydryls are not present in sufficient quantities on the second molecule, they can be introduced with Traut's Reagent (2-iminothiolane, UP42425A) or *N*-succinimidyl *S*-acetylthioacetate (SATA, UP84235A) ny reaction on amides. Alternatively, they can be generated through reduction of disulfides with mercaptoethylamine•HCl., or DTT (UP284250), or TCEP (UP242214).

- The **hydrazide** group reacts specifically with aldehyde, forming a stable hydrazone bond.
  R-CHO + N_2-NH-R' → R-CH=N-NH-R'

Aldhehydes 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 $NaIO_4$ at RT in the dark (Chamow 1978):
R-CH(OH)-CH(OH)-R + $NaIO_4$ → R-CHO

Rem: Hydrazide also reacts with carboxylic acids in the presence of EDAC (#UP520050)
R-COOH + Hydrazide-R' + EDAC → R-CO-NH-NH-CO-$(CH_2)_4$-R'

As hydrazone bond is not enough stable at very low pH, this can be converted to hydrazine by reaction with $NaHBH_4$ for some applications (O'Shannessey 1990).

- **MPS-EDTA** is a unique sulfhydryl and carbonyl reactivity heterobifunctional crosslinker. It is water soluble, and reacts to amine with active ester by neutralizing with a hindered 3° amine, e.g., 2,6-lutidine (one that will not attack the maleimide). Followed by sulfhydryl coupling to the maleimide.

- 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 perform the reaction to carbohydrate through the hydrazide. Desalting of conjugates (to remove by products, change buffer) may be performed by dialysis (CelluSep) or gelfiltration – see related products-.

When a molecule bears both chemical groups, dimers may form. To avoid this, one may block undesired SH on the carbonyl bearing molecule for the activation step. 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 re-oxidation of SH into disulfides (Ishikawa 1983).

Antibody oriented conjugation(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).

References

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-

Other information

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

Related products:

*Other amino group modifyers:
▪2-Iminothiolane (**Traut's reagent**) #UP42425A to convert amino group to un-protected sulfhydryl groups
▪SulfoSuccinimidyl-Acetate (**SulfoNHS-Acetate**) #UP69380A to block amino groups
▪ 6-(N-trifluoroacetyl)caproic acid NHS (**TFCS**) #L7727B to protect amino group that can then be unmasked at pH 7.8-8.1
▪Succinimidyl-p-formyl-benzoate (**SFB**) #M11771 to convert amino group in CHO groups
▪Labeling amino groups: for conversion of amine - in biotin tether (labeling), i.e. NHS-Biotins i.e. NHS-PEO4-Biotin UPR2027A,
- or to fluorescent moiety, i.e. NHS-FluoProbes
▪conversion to reactive groups: see below bifunctional crosslinkers

*Sulfhydryls modifyers:
▪Pyridine dithioethylamine hydrochloride (PDA) #BI1321 to converts sulfhydryl group in amino group.
▪Labeling sulfhydryl groups: conversion of SH -in biotin tether (labeling), i.e. Maleimide-Biotins i.e. MAL-PEO-Biotin #R2028A
-or to fluorescent moiety, i.e. Maleimide-FluoProbes

*Other crosslinkers:
▪Heterobifunctional crosslinkers: Maleimido-NHS reagents (i.e. SMCC), photoActivable / amine reactive (i.e. SCBP #BI1361),...
▪Biotin Labeling : NHS-Biotins i.e. NHS-PEO4-Biotin UPR2027A, Maleimide-Biotins
▪Fluorescent Labeling : FluoProbes chromophores labeling agents (NHS, Maleimide, Hydrazide...)

▪Homobifunctional crosslinkers:
NHS-NHS reagents, i.e. NHS-PEO-NHS and SMCC #54940A #AL6580
MAL-MAL reagents, i.e. MAL-PEO-MAL #L7736A
▪PhotoActivable (PA) crosslinkers: SH and PA reactive i.e. SCBP #BI1361,...

▪Heterobifunctional crosslinkers:
MAL-NHS reagents, i.e. MAL-PEO-NHS

*Desalting tools: ▪CelluSep dialysis tubings ▪Desalting gelfiltration columns #UP84874

*Carrier proteins: ▪KLH UP88502, BSA, OVA MaxiBind proteins

see BioSciences Innovations catalogue and e-search tool.

Catalog size quantities and prices may be found at http://www.interchim.com.
Please inquire for higher quantities (availability, shipment conditions).
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