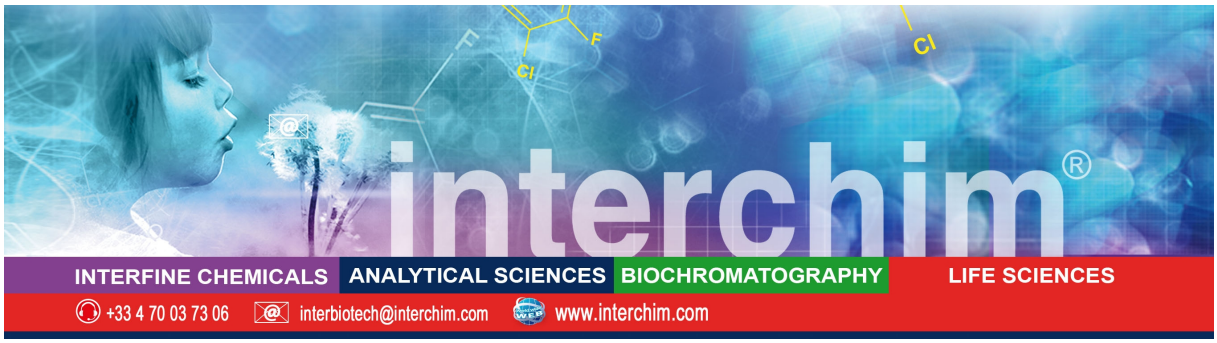
FT-UP09833

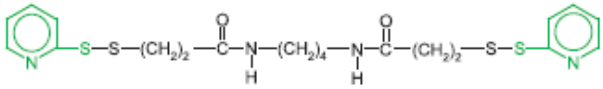


Cleavable SH-reactive crosslinkers DPDPB, DTME, MMP

Products Description

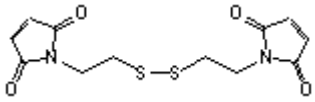
cleavable homobifunctional Sulfhydryls (SH) reactive crosslinkers

Catalog nb: UP09833A, 100mg UP09833B, 50mg
Name: **DPDPB (Lomant's reagent)**



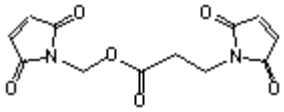
Formula : 1.4-Di(3'-(2'-pyridylthio)propionamido)butane
M.W.= 482.71 - Spacer: 19.9 Å
Cleavable with reducing agents such as DTT, bME,…

Catalog nb: L7734A, 50mg
Name: **DTME**



Formula : Dithio-bis-maleimidoethane; (DTME)
M.W.= 312.37 - spacer is 13.3 long
Cleavable with reducing agents such as DTT, bME,…
1. Han, J.C., et.al. (1994) Anal. Biochem. 220, 5-10.

Catalog nb: BJ005A, 50mg
Name: **MMP**



Formula : Maleimidopropionic acid maleimidomethyl ester
M.W.= 278.22
Cleavable with 0.02 M hydroxylamine or mild base.
Useful for crosslinking substrates to erythrocyte membranes.1
1. Sato, S., et.al. (1981) J. Biochem. 1177-1185.

Storage: Store at +4°C protected from light and moisture (L)

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 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…). Considering the desired result, one should choose adequate chemical reactivity and to the nature and length of the spacer.

Uptima offers a high quality crosslinker to answer the needs of coupling proteins and peptides for various biotechnologies as well R&D studies or (other cross-linkers are available). DPDB and BMOE elicits a reactivity toward sulfhydryls.

- Obtention of immunogens 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…

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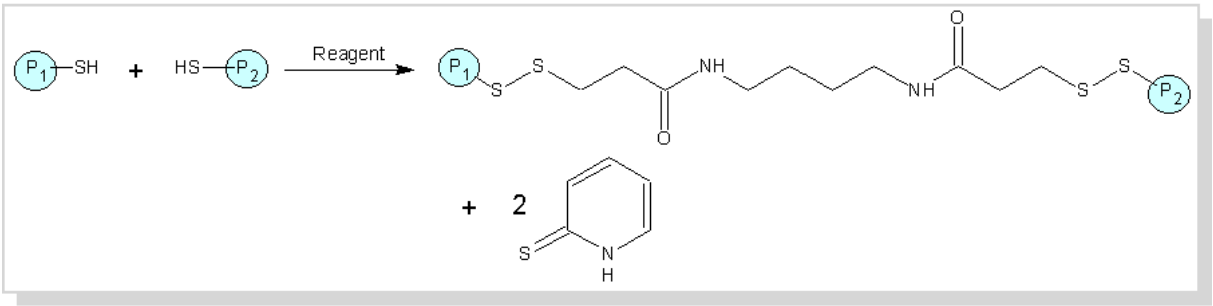
- Obtention of oligomeric conjugates : conjugates of oriented peptides for immunization, dimeric proteins for structural studies, grafting haptens onto cells…
- Obtention of biologically active conjugates: specific antibody coupled to drugs for immunotargetting techniques, immunotoxins, …
- Modification of proteins for R&D studies, as ribosomes (Zecherle 1992) , replication enzymes (Latham 1999) .

See related products for **Analogs with alkyl spacer** (BMOE, BMB and BMH)
Analogs with a cleavable spacer (HBVS, DTME, DPDPB/Loment, MMP)

Scientific and technical information

- Open the vial when it has reached room temperature only. DPDPB is soluble in DMSO, DMF, Methanol (>80mM) and Dioxane, Ethanol (>20mM), but quite insoluble in water.
- The **pyridyl thiol** group reacts specifically at pH7-9 by exchange with sulfhydryl, leaving a pyridine-2-thione group that can be followed up: maximum absorbtion occurs at 343nm with an extinction coefficient of 8.03 $.10^3$ M^{-1} cm^{-1} (Struchbury 1975). The formed link includes a –S-S- bound (disulfide). Classic conditions of reaction are 30min at +25-37°C. An excess of crosslinker is generally to activate the first protein, then the excess is removed to allow conjugation to the secund protein at an equi-molar ratio.

Cys-SH



- The spacer measures 1.6nm (10Angstroms) in DPDB, and 9.9 Å in BMOE. DPDB spacer is easily cleaved with a reducing agent such as DTT.
- Proteins containing disulfide bonds can be reduced by DTT (#UP28425) at pH 7-9 for 30min to generate SH before coupling to pyridylthiol group, or SH can be introduced thanks 2-Iminothiolane (#UP42425) or SATA (#UP84235). Excess of DTT should naturally be removed before reaction with DPDPB abd BMO reagents.
- The maleimide group reacts very specifically with sulfhydryls –SH at neutral pH 6.5-7.5. The reaction is rapid (a few minutes for cystein), but may require 1-2 hours to be completed in certain conditions. The competiting hydrolysis forming maleamic acid becomes noticeable when pH go up 8.0, where the reactivity with amines begins to be possible. It is stable in 0.1 M phosphate, pH 7.0, 4 °C, for 64 h (Yoshitake 1979) . In usual conditions, 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 almost 1:1 coupling.

Directions for use

Protocol 1: coupling proteins with DPDPB

- For coupling procedures, typically, prepare a 10-25mg/ml working solution in DMSO. Uptima recommends not to store it.
- The first protein1 to be coupled is prepared in PBS(NaCl 150mM, phosphate 20mM, pH7.2) or other physiological buffers, provided there is no free sulfhydryl. SH may have been introduced by mild reduction of dissulfide bridges (i.e. in IgG antibodies) with DTT or 5mg/ml 2-mercapthoethylamine in pH 6.0 buffer for 2H at 37°C, then desalted.
- DPDB is added to molar ratios of 50-100 per protein molecule (IgG). Incubate for for 30min at +37°C.

- Desalt (Use CelluSep membranes or FastDialysers) the activated protein1
- Add one mole of the second protein2 to be coupled for each mole of activated protein1, and allow to react for 30min at +37°C.
- Exchange buffer for storage or further use of the conjugate.

Notes: incubation temperature may be decreased if the proteins are sensible, to +30°C or lower but incubation duration should then be increased.

Literature for DPDPB

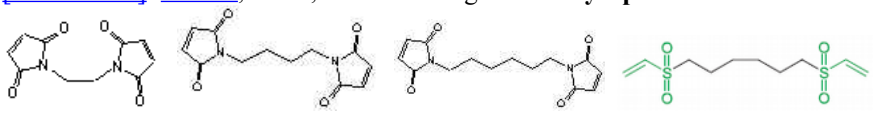
Chen 1995: Chen, L.L., Frankel, A.D. Harder, J.L., Fawell, S., Barsoum, J. and Pepinsky, B. (1995). Increased cellular uptake of the human immunodeficiency virus-1 tat protein after modification with biotin. Anal. Biochem. 227, 168-175. UP0933
Latham 1999: Latham G. J., Feng Dong, Pietroni P., Dozono J.M., Bacheller D.J., Von Hippel P.H.; Opening a monomer-monomer Interface of the trimeric bacteriophage T4 code GP45 sliding clamp is required for clamp loading onto DNA; PNAS (1999), vol.96, n°22, 12448-12453 UP09833
Zecherle (1992): Zecherle GN, Oleinikov A, Traut RR. ; The proximity of the C-terminal domain of Escherichia coli ribosomal protein L7/L12 to L10 determined by cysteine site-directed mutagenesis and protein-protein cross-linking.J Biol Chem 1992 Mar 25;267(9):5889-96 UP09833

Literature for BMOE

Yoshitake, S., et.al. (1979) *Eur. J. Biochem.* 101, 395-399.

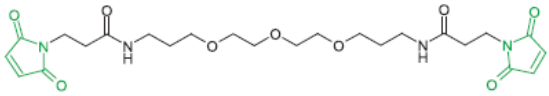
Related products

● **Analogs** homobifunctional crosslinkers, with alkyl spacer or cleavable spacers:
[FT-L7730A]: BMOE, BMB, HBSV: analogs with **alkyl spacer**



[FT-L7734A]: DPDPB, DTME, MMP: analogs with **cleavable**

[FT-L7736A]: MAL-PEO-MAL: **hydrophilic spacer** analogs of BMOE, BMB, BMH



[FT-BJ002A]: BMP2 and NMP3: analogs with **aromatic** spacer analogs

● **Hetero**bifunctional Crosslinkers
[FT-AZ4170]: MAL-PEO-COOH: analogs with Maleimido **and Acids** reactive groups, and with hydrophilic spacer
And many others…

- Heterobifunctional crosslinkers, Amines & Sulfhydryls reactive, i.e. NHS-PEO-MAL (AL6581) and SMCC (17412A)
- Homobifunctional crosslinkers: Amines reactive, i.e. NHS-PEO-NHS (BH8811) and DSS (54940A)
  Sulfhydryls reactive, i.e. MAL-PEO-MAL (L7736A) and BMOE (L7730A)
- Hydrazone chemistry: Conjugation kit (BL1501) and HynNic crosslinkers (SANH #BL9270, MHPH #BL9401 SH-reactive)

●Other products using BioSciences Innovations catalogue and e-search tool.

Additional information

For R&D in vitro use only

For any question, please contact Uptima or your local distributor
e-mail Uptima@interchim.com; Hotline : +33(0)4 70 03 76 06

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