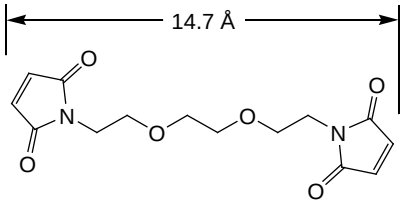
INSTRUCTIONS

$BM(PEG)_2$ and $BM(PEG)_3$

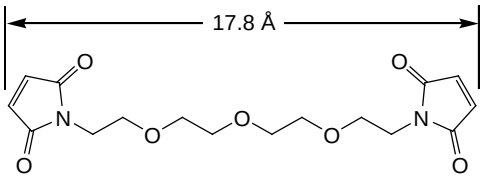
22336 22337

0786.5

Number	Description
22336	**$BM(PEG)_2$,** 1,8-bis(maleimido)diethylene glycol, 50mg Molecular Weight: 308.29 Spacer Arm: 14.7Å Net Mass Added: 308.10



22337	**$BM(PEG)_3$,** 1,11-bis(maleimido)triethylene glycol, 50mg Molecular Weight: 352.34 Spacer Arm: 17.8Å Net Mass Added: 352.13



Storage: Upon receipt store desiccated at 4°C. Product is shipped at ambient temperature.

Introduction

Thermo Scientific $BM(PEG)_2$ and $BM(PEG)_3$ are homobifunctional, maleimide crosslinkers for conjugation between sulfhydryl groups (-SH). Such bismaleimide crosslinkers are commonly used to explore and characterize protein structure (i.e. oligomerization) or protein interactions. Because $BM(PEG)_2$ and $BM(PEG)_3$ have the same reactivity but differ in length, the relative success of these and similar reagents in forming crosslinks between sites in a protein oligomer or interaction can assist in determining intra- and intermolecular distances. (See the Related Products section at the end of these instructions for a list of other bismaleimide crosslinkers.)

$BM(PEG)_2$ and $BM(PEG)_3$ are unique among bismaleimide crosslinkers in having spacer arms with hydrophilic polyethylene glycol (PEG) groups, also called polyethylene oxide (PEO). This type of spacer imparts better water solubility to the reagent and conjugates compared to hydrocarbon spacers of other reagents.

Reaction of a sulfhydryl to the maleimide group results in formation of a stable thioether linkage (Figure 1), which cannot be cleaved by reducing agents or physiological buffer conditions. Reaction of maleimides is very specific to sulfhydryls at pH 6.5-7.5.[1] Although maleimides will react to primary amines at pH >8, the rate is 1000 times slower than the reaction to sulfhydryls at pH 7. Unlike iodoacetamides, maleimides do not react with tyrosines, histidines or methionines.

The maleimide moiety is temporarily stable in aqueous solutions devoid of reactive sulfhydryl targets, but hydrolysis to a nonreactive maleamic acid can occur during storage, especially at pH >8 (Figure 1). For this reason, dissolved reagents are best used promptly and the remainder discarded. Hydrolysis of the ring structure also can occur following conjugation, resulting in an open-ring linkage[2] (Figure 1).

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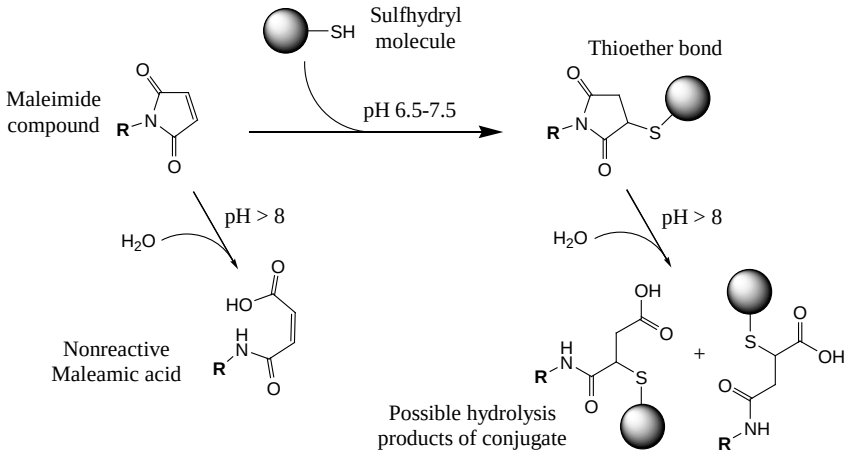

Figure 1. Reaction of maleimide-activated compounds to sulfhydryls.

Important Product Information

- Molecules to be reacted with maleimide compounds must have free (reduced) sulfhydryls. Reduce peptide disulfide bonds with Thermo Scientific Immobilized TCEP Disulfide Reducing Gel (Product No. 77712). Reduce disulfide bonds in high molecular weight proteins using 5mM TCEP (1:100 dilution of Thermo Scientific Bond-Breaker TCEP Solution, Product No. 77720) for 30 minutes at room temperature, followed by TCEP removal using a desalting column (e.g., Thermo Scientific Zeba Spin Desalting Columns). Proteins (e.g., antibodies) can be inactivated by complete reduction of their disulfide bonds. Selective reduction of hinge-region disulfide bonds in IgG can be accomplished with 2-Mercaptoethylamine•HCl (2-MEA, Product No. 20408). Sulfhydryls can be added to molecules using *N*-succinimidyl *S*-acetylthioacetate (SATA, Product No. 26102) or 2-iminothiolane•HCl (Traut's Reagent, Product No. 26101), which modify primary amines.
- Avoid extraneous sulfhydryl-containing components in the reaction buffers during conjugation (e.g., DTT), as they react with the maleimide portion of the reagent, inhibiting and reducing conjugation efficiency of the intended target.
- The maleimide group reacts predominantly with free sulfhydryls at pH 6.5-7.5, forming stable thioether bonds. At pH values >7.5, reactivity toward primary amines and hydrolysis of the maleimide groups can occur. At pH 7, the maleimide group is ~1000 times more reactive toward a free sulfhydryl than to an amine.

Procedure for Crosslinking Proteins in Solution

Generally, a two- or three-fold molar excess of crosslinker over the amount of sulfhydryl-containing protein(s) results in sufficient conjugation between proximal sulfhydryl groups. Empirical testing of reagent and protein concentrations is necessary to determine optimal conditions for the experiment.

A. Material Preparation

- Conjugation Buffer: Phosphate buffered saline (PBS, pH 7.2; e.g., Product No. 28372) or other sulfhydryl-free buffer at pH 6.5-7.5. Include 5-10 mM EDTA to help prevent the reoxidation of disulfides by trace divalent metals.
- Crosslinker Stock Solution: Immediately before use, weigh a small quantity of crosslinker and dissolve it in dimethylformamide (DMF) or dimethylsulfoxide (DMSO) at a 5-20mM concentration. For example, make a 20mM solution of each reagent as follows:
    - For $BM(PEG)_2$, dissolve 3.1mg reagent in 0.5mL DMF or DMSO
    - For $BM(PEG)_3$, dissolve 3.5mg reagent in 0.5mL DMF or DMSO

  Note: Alternatively, reagent can be dissolved at <10mg/mL in warm (37°C) water in about 10 minutes. Warming the crosslinker will not affect the integrity of the maleimide groups.
- Sulfhydryl-containing protein, prepared as described the Important Product Information section.
- (Optional): Quenching Solution: concentrated (0.5-1M) cysteine, DTT, or other thiol-containing reducing agent.
- (Optional): Desalting column (e.g., Zeba™ Spin Desalting Columns) or dialysis unit (e.g., Thermo Scientific Slide-A-Lyzer Dialysis Cassettes) to separate crosslinked proteins from excess nonreacted crosslinker.

B. Procedure for Protein Crosslinking

1. Dissolve protein(s) in Conjugation Buffer at 0.1mM (e.g., 5mg in 1mL for a 50kDa protein).
2. Add crosslinker to the dissolved protein(s) at 0.2mM final concentration (= two-fold molar excess for 0.1mM protein solution) by adding 10µL of Crosslinker Stock Solution per milliliter of protein solution.

   Note: The reaction solution may appear cloudy as a result of the low aqueous solubility of the crosslinker; usually, such solutions become clearer as the reaction proceeds. However, initial solubility can be increased by gentle heating and sonication. Other concentrations of Crosslinker Stock Solution can be used, as well as other final molar fold excesses of crosslinker. Many proteins will precipitate when the DMF or DMSO concentration exceeds 10-15% of the final reaction volume; if protein solubility is not an issue, there is no limit to the DMF or DMSO concentration that may be used.
3. Incubate reaction mixture for 1 hour at room temperature or for 2 hours at 4°C.
4. Quench reaction by adding Quenching Solution at 10-50mM final and incubating for 15 minutes at room temperature. Alternatively (or in addition) remove the excess nonreacted reagent by desalting or dialysis.

Related Thermo Scientific Products

Table 1. Bismaleimide crosslinkers.

Crosslinker Name	Spacer Arm Length (Å)	Spacer Arm Composition (between maleimide groups)	Product No.
BMOE	8.0	Alkane	22323
BMDB	10.2	Cis-diol (periodate cleavable)	22332
BMB	10.9	Alkane	22331
BMH	13.0	Alkane	22330
DTME	13.3	Disulfide (reducing agent cleavable)	22335
$BM(PEG)_2$	14.7	Polyethylene glycol (PEG)	22336
$BM(PEG)_3$	17.8	Polyethylene glycol (PEG)	22337

Cited References

1. Smyth, D.G., Blumenfeld, O.O. and Konigsberg, W. (1964). Reaction of *N*-ethylmaleimide with peptides and amino acids. *Biochem J* **91:**589.
2. Partis, M.D., *et al.* (1983). Crosslinking of protein by w-maleimido alkanoyl *N*-hydroxysuccinimido esters. *J Protein Chem* **2(3):**263-77.

Product References

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Green, N.S., Reisler, E. and Houk, K.N. (2001). Quantitative evaluation of the lengths of homobifunctional protein cross-linking reagents used as molecular rulers. *Protein Sci* **10:**1293-304.

Jastrzebska, B., *et al.* (2004). Functional characterization of the rhodopsin monomers and dimers in detergents. *J Biol Chem* **279(52):**54663-75.

Lim, C-J. and Shen, W-C. (2004). Transferrin-oligomers as potential carriers in anticancer drug delivery. *Pharmaceut Res* **21(11):**1985-92.

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