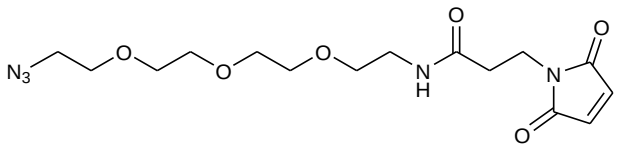
Azido-PEG3-Maleimide (BP-22468)

Product Name: Azido-PEG3-Maleimide

Catalog number: BP-22468

Chemical Structure:



Formular: $C_{15}H_{23}N_5O_6$

Molecular Weight: 369.4

Solubility: DMSO, DMF, DCM

Appearance: Vial 1 Off-white to grey solid; Vial 2 Slightly yellow oil

Storage: Upon receipt store at -20°C. Product shipped at ambient temperature

Shelf life: for each component, at least 12 months at -20°C

Important Note: *Azido-PEG3-Maleimide degrades quickly (hours) at room temperature. This product is provided as a two-component kit. The stock solution of Azido-PEG3-Maleimide is prepared in situ.*

Introduction

Azido-PEG3-maleimide is mainly used to crosslink two biomolecules together. The maleimide reacts with thiol-containing compound at pH 6.5 to 7.0 to activate the molecules, then the azide will react with alkyne-containing compound to yield the conjugates.

Note:

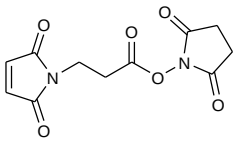
- pH for the thiol-maleimide reaction: 6.5-7.5.
  The maleimide group reacts predominantly with free sulfhydryls to form thioether. At pH > 7.0, primary amines will also react with maleimide, and hydrolysis of the maleimide groups will occur. At pH 7, the maleimide group is ~1,000 times more reactive toward a free sulfhydryl than to an amine.
- Thiol and azide free: no thiol and azide residual in biomolecules and buffers.

Additional Materials Required

- Co-solvent: dimethyl sulfoxide (DMSO) or dimethyl formamide (DMF)
- Reducing reagents: TCEPT or Immobilized TCEP disulfide reducing gel
- Reaction buffer: PBS buffer (pH 6.5-7.0) with 5-10 mM EDTA
- (Optional) quenching buffer: concentrated (0.5-1 M) cysteine, DDT or other thiol containing reducing agents
- Spin Desalting Columns

Preparation of Azido-PEG3-Maleimide Stock Solution

Vial 1



BP-20509

721mg

$C_{11}H_{10}N_2O_6$
MW: 266.21

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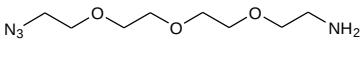
Vial 2



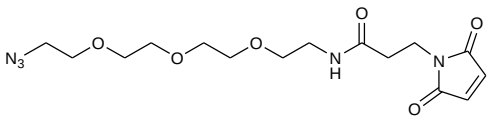
BP-20580

600mg

$C_8H_{21}N_4O_3$
MW; 221.28

→

BP-22468



1g

$C_{15}H_{23}N_5O_6$
Mol. Wt.: 369.37

1. Add 2.5ml of dry DMF or DMSO to Azido-PEG3-amine (vial #2) and shake for ~30 seconds.
2. Add the solution above slowly to maleimide-NHS ester (vial #1, white solid) under a dry N2 atmosphere slowly, stir for 30 minutes at room temperature. The progress of the reaction can be followed by TLC.
3. Stock solution of Azido-PEG3-Maleimide is ready to use. At this stage the product is stable if stored at -20C or lower for short periods of time (hours).
4. The concentration of azide-PEG3-maleimide stock solution is about 120 mM, i.e. is amount: 0.3 mmol.
5. TLC data: methanol: methylene chloride 1:20 or 4 ml: 10 drops, silica gel normal phase plate developed with a potassium permanganate spray.
   E.g. the Rf of the Azido-PEG3-Maleimide is slightly lower one of the maleimide-NHS ester. When the reaction is complete, it will be one clean spot on the plate.

Procedure for Labeling Proteins

1. If required, buffer exchange the protein sample into PBS at 1-5 mg/mL by using a spin desalting column.
2. Add TCEP stock solution to the protein solution at final concentration of 20 mM, pipette up and down several times to mix.
3. Incubate the reaction for 30 minutes.
4. Buffer exchange into reaction buffer to remove excess TCEP.
   Note: EDTA need be added to reaction buffer to a final 5-10 mM to avoid S-S bind reformation.
5. Add a 20 x freshly prepared maleimide reagent above to the protein sample.
6. Incubate reaction mixture for 1-4 hour at room temperature or for 2-8 hours at 4°C.
   Note: many proteins will precipitate when the DMF or DMSO concentration exceeds 10% of the final

reaction volume.

7. Remove the excess reagent by desalting the labeled protein through a spin desalting column or by dialysis.

Procedure for click reaction

Please check a corresponding protocol in the **PEG linkers for click chemistry** for details.