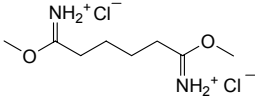
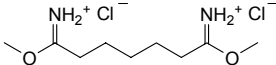
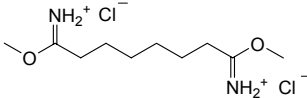
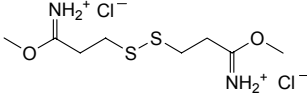


Imidoester Crosslinkers: DMA, DMP, DMS, DTBP

0668.2

Number	Description	
20660	DMA (Dimethyl adipimidate•2 HCl) , 1 g Molecular Weight: 245.15 Spacer Arm Length: 8.6 Å	
21667 21666	DMP (Dimethyl pimelimidate•2 HCl) , 1 g DMP (Dimethyl pimelimidate•2 HCl) , 50 mg Molecular Weight: 259.17 Spacer Arm Length: 9.2 Å	
20700	DMS (Dimethyl suberimidate•2 HCl) , 1 g Molecular Weight: 273.20 Spacer Arm Length: 11.0 Å	
20665	DTBP (Dimethyl 3,3'-dithiobispropionimidate•2HCl) , 1 g Molecular Weight: 309.28 Spacer Arm Length: 11.9 Å	

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

Introduction

DMA, DMP, DMS and DTBP are water soluble, membrane permeable, homobifunctional imidoester crosslinkers. The imidoester functional group is one of the most specific acylating groups available for the modification of primary amines and has minimal cross reactivity toward other nucleophilic groups in proteins.^{1,2} In addition, the imidoamide reaction product does not alter the overall charge of the protein, potentially retaining the native conformation and activity of the protein.

Important Product Information

- Imidoester crosslinkers are moisture sensitive. To avoid condensation onto the product, fully equilibrate vial to room temperature before opening (typically requires at least 30 minutes).
- For imidoester crosslinking reactions use buffers such as phosphate, borate, carbonate and HEPES that do not contain primary amines. Imidoesters react with amines at pH 7-10. For optimal crosslinking efficiency, use pH 8-9.
- Imidoester crosslinkers cannot be stored in solution because the imidate moiety is easily hydrolyzed.
- DMA, DMP and DMS are non-cleavable forms of imidoester crosslinkers. By contrast, crosslinks with DTBP can be cleaved by reducing the disulfide bond of the spacer arm with 100-150 mM DTT at 37°C for 30 minutes.

General Procedure for Crosslinking Proteins

The following protocol is adapted from a procedure described by Packman and Perham.³

A. Materials Required

- Crosslinking Buffer: 0.2 M triethanolamine, pH 8.0. Do not use buffers that contain primary amines (e.g., Tris, glycine, etc.), as these buffers will compete with the crosslinking reaction.
- Stop Solution: Glacial acetic acid. Alternatively, Tris or glycine can be used to quench the reaction.

B. Procedure

1. Prepare the appropriate protein sample in crosslinking buffer.
2. Add a 10-fold molar excess of the cross-linker to the protein when the protein concentration is above 5 mg/ml. If the protein concentration is below 5 mg/ml add a 20- to 30-fold molar excess of the crosslinker.
3. Incubate the reaction at room temperature for 30-60 minutes.
4. Add glacial acetic acid at a 1:4 ratio to the sample to stop the reaction. Alternatively, stop the reaction by adding Tris or glycine at a 20-50 mM final concentration.

Related Thermo Scientific Products

20036	Bioconjugate Techniques (Book), 1202 pages, softcover
28384	BupH™ Borate Buffer Packs , 40 packs (each makes 500 ml of pH 8.5 buffer)
28372	BupH Phosphate Buffered Saline Packs , 40 packs (each makes 500 ml of pH 7.2 buffer)
20291	DTT, No-Weigh™ Format , 48 x 7.7 mg microtubes (each makes 500 mM solution upon addition of 100 µl of water)

References

1. Hand, E.S., and Jencks, W.P. (1962). Mechanism of the reaction of imidoesters with amines. *J. Am. Chem. Soc.* **84**: 3505-14.
2. Mattson, G., Conklin, E., Desai, S., Nielander, G., Savage, D., and Morgensen, S. (1993). A practical approach to crosslinking. *Mol. Biol. Rep.* **17**: 167-83.
3. Packman, L.C. and Perhan, R.N. (1982). Quaternary structures the pyruvate dehydrogenase multienzyme complex of *Bacillus stearothermophilus* studies by a new reversible crosslinking procedure with bis(imidoesters). *Biochem.* **21**: 5171-5.
4. Cole, N.B., *et al.* (2002). Lipid droplet binding and oligomerization of the Parkinson's disease protein alpha-synuclein. *J. Biol. Chem.* **277**: 6344-52.
5. Deleault, N., *et al.* (2005). Protease-resistant prion protein amplification reconstituted with partially purified substrates and synthetic polyanions. *J. Biol. Chem.* **280**: 26873-9.
6. Chang, Z., *et al.* (2004). Chemical crosslinking and spectrometric identification of sites of interaction for UreD, UreF and urease. *J. Biol. Chem.* **279**: 15305-13.
7. Mikhailov, V., *et al.* (2001). Bcl-2 prevents bax oligomerization in the mitochondrial outer membrane. *J. Biol. Chem.* **276**: 18361-74.

This product ("Product") is warranted to operate or perform substantially in conformance with published Product specifications in effect at the time of sale, as set forth in the Product documentation, specifications and/or accompanying package inserts ("Documentation") and to be free from defects in material and workmanship. Unless otherwise expressly authorized in writing, Products are supplied for research use only. No claim of suitability for use in applications regulated by FDA is made. The warranty provided herein is valid only when used by properly trained individuals. Unless otherwise stated in the Documentation, this warranty is limited to one year from date of shipment when the Product is subjected to normal, proper and intended usage. This warranty does not extend to anyone other than the original purchaser of the Product ("Buyer").

No other warranties, express or implied, are granted, including without limitation, implied warranties of merchantability, fitness for any particular purpose, or non infringement. Buyer's exclusive remedy for non-conforming Products during the warranty period is limited to replacement of or refund for the non-conforming Product(s).

There is no obligation to replace Products as the result of (i) accident, disaster or event of force majeure, (ii) misuse, fault or negligence of or by Buyer, (iii) use of the Products in a manner for which they were not designed, or (iv) improper storage and handling of the Products.

Current versions of product instructions are available at www.thermo.com/pierce. For a faxed copy, call 800-874-3723 or contact your local distributor.

© 2009 Thermo Fisher Scientific Inc. All rights reserved. Unless otherwise indicated, all trademarks are property of Thermo Fisher Scientific Inc. and its subsidiaries. Printed in the USA.