

# Homobifunctional cross-linkers

## DSS, BS3, DSG, NHS-PEO<sub>x</sub>-NHS

### Products Description

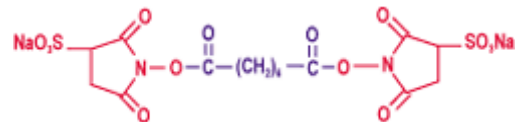
DSS and its hydrosoluble derivate BS3 are popular homobifunctional crosslinkers.

Their analogs with PEO<sub>x</sub> spacer, also called BS(PEG<sub>x</sub>) or Bis-Succinimidyl-dPEG<sub>x</sub>, display hydrosolubility and improved features.

- Alkylated spacer versions

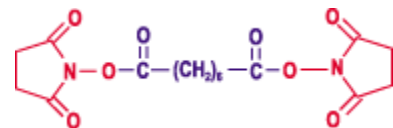
**Catalog number:** 549408, 10 mg    549409, 50 mg  
 UP54940A, 100 mg    UP54940B, 250 mg  
 54940C, 1g

**Name:** **BS<sub>3</sub> (Sulfo-DSS)**  
 Bis-(Sulfo-succinimidyl)-Suberate; CAS: 82436-77-9  
 MW= 572.4 . Spacer 11.4 A



**Catalog number:** UP28065A, 1 g    UP28065B, 5x1 g

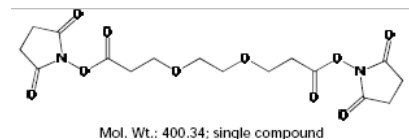
**Name:** **DSS**  
 DiSuccinimidyl Suberate; CAS: 68528-80-3  
 MW= 368.4 . Spacer 11.4 A



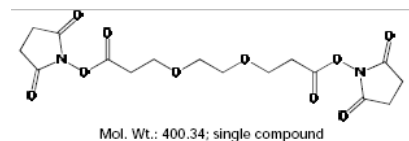
**Storage :** -20°C (possible at 4°C) , protect from moisture and light. (M)

- PEGylated spacer versions

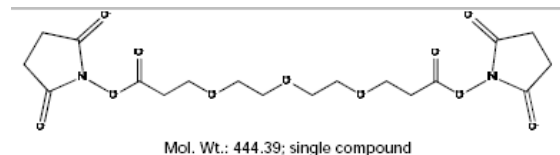
**Catalog number:** BM3011, 100mg    BM3013, 1g  
**Name:** **NHS-PEO<sub>2</sub>-NHS**  
 MW= 400.34  
 Spacer 11.0A (10atoms)



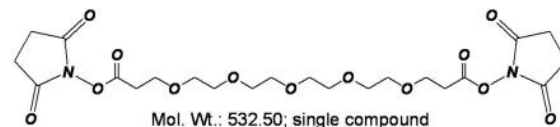
**Catalog number:** BM3011, 100mg    BM3013, 1g  
**Name:** **NHS-PEO<sub>2</sub>-NHS**  
 MW= 400.34  
 Spacer 11.0A (10atoms)



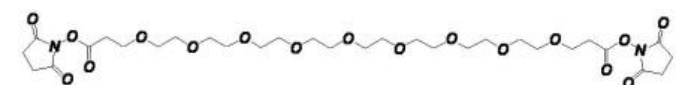
**Catalog number:** FL8551, 100 mg    FL8553, 1g  
**Name:** **NHS-PEO<sub>3</sub>-NHS**  
 MW= 444.39  
 Spacer 14.6A (13atoms)



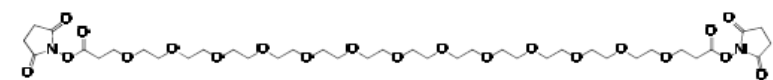
**Catalog number:** BH8811, 100 mg    BH8813, 1g  
**Name:** **NHS-PEO<sub>5</sub>-NHS**  
 MW= 532.50; CAS:756526-03-1  
 Spacer 21.7A (19atoms)



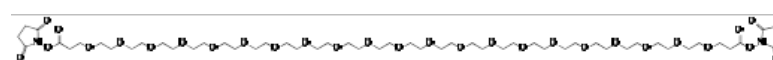
**Catalog number:** CQ2051, 100 mg    CQ2053, 1g  
**Name:** **NHS-PEO<sub>9</sub>-NHS**  
 MW= 708.71-685.71  
 Spacer 35.8A (31atoms)



**Catalog number:** FL8561, 100 mg    FL8563, 1g  
**Name:** **NHS-PEO<sub>13</sub>-NHS**  
 MW= 884.92<sup>(K)</sup>  
 Spacer 50.1A (43atoms)



**Catalog number:** FL8571, 100 mg    FL8573, 1g



FT-54940A

Name:

**NHS-PEO<sub>21</sub>-NHS**

MW= 1237.34

Spacer 79.1 Å (67atoms)

## Scientific and Technical Information

Cross-linkers are chemical reagents used to conjugate molecules together by a covalent bond. 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...). The choice of the crosslinker to design of the right conjugate is driven notably by the reactivities, and the nature and length of the spacer.

Homobifunctional cross-linkers present 2 identical reactivities. In this family, **DSS** has been popularized. It reacts toward amines, through succinimide groups, and is non-cleavable. Uptima offers a high quality DSS, its sulfonated form (BS3), a longer spacer version (DSG) and a series of analogs with PEO spacers lengths, for great benefits over conventional DSS (solubilisation, conjugates more hydrophilic, biocompatibility,...). These reagents answer the needs of coupling proteins and peptides for biological and immunoassays like (other cross-linkers are available):

- Obtention of immunogen carrier-hapten
- Obtention of biologically active conjugates
- Studies of di- or oligo-meric proteins for structural investigations
- Coating of polystyrene surfaces for immunoassay
- Grafting haptens onto cells and particles (beads) for diagnostics...
- Obtention of di-specific affine probes
- Obtention of oligomeric conjugates : poly- peptides
- Grafting peptides onto gels for chromatography separations

### Chemical features:

- The chemical group **N-hydroxysuccinimydyl** (NHS) reacts in aqueous phase on primary ( $-NH_2$ ) and secondary amines ( $=NH$ ) (in fact on its deprotonated form), optimally at neutral pH or higher : amines present in proteins (Lys aminoacid) and in a lower proportion on  $NH_2$  located in terminal peptidic chains. Higher the pH increases, and higher the dilution of the molecule is, more the reaction competes with hydrolysis.
- The **sulfonyl moiety** ( $NaSO_3$ ) introduces a hydrophilic group, that allows the product not to cross biological membranes. An other interest of the sulfonyl group is to permit the solubilisation of the product directly in aqueous buffers, up to 10 mM, avoiding the use of organic solvents like DMSO or DMF, which are possibly nocive to cells or applications.
- The **spacer** arms of DSS and BS<sub>3</sub> are 8 atoms long, for a length of 11.4 Angstroms
- The **spacer arm** of PEO products have PolyEthylOxy (= PolyEthylenGlycol: PEG) structure which is hydrophilic. The spacer-mediated hydrophilicity is a superior alternative to sulfonyl moiety derivatives of NHS: PEO products are not only water-soluble, but also confers hydrophilicity to the formed conjugates! Different lengths are available, increasing the hydrophilicity, and the flexibility of the spacer.

## Directions for Use

### Solubilisation (immediately before use)

Allow vial of BS3 Crosslinker to fully equilibrate to ambient temperature before opening to prevent condensation . Dissolve BS3 first in water or 20mM sodium phosphate buffer, 10mM concentration. Although solubility is up 5.8mg/ml in water, solubilization may be affected by more concentrated salts, but dissolved BS3 can be further diluted with more concentrated buffer solutions.

Prepare DSS by dissolving in DMSO or DMF. Use dry dimethyl sulfoxide (DMSO) or dimethyl formamide (DMF)

FT-54940A

## Protocol<sup>F</sup>: Protein conjugation using BS3

### 1. Prepare buffers

• Conjugation Buffer: Use a buffer free of amines at pH 7 to 9, such as 100mM sodium phosphate, 0.15M NaCl ); 20mM HEPES; 100mM carbonate/bicarbonate; or 50mM borate.

### 2. Prepare crosslinker immediately before use. See above section.

### 3. Add crosslinker to the protein sample. Typical final concentration of crosslinker is 0.25 - 5mM.

Use a 10-fold (10:1 Crosslinker:Protein) molar excess of the crosslinker for protein > 5mg/mL

Use a 20- to 50-fold molar excess of the crosslinker over protein at < 5mg/mL

Note: The conjugation of a protein or a mix of proteins lead to a variety of conjugates species and sizes, depending also on the ratio of proteins and of crosslinker.

For conjugating 2 different proteins, an oriented conjugation strategy is recommended, such a SMCC based.

### 4. Incubate the reaction mixture at room temperature for 30-60 minutes, or on ice for 2 hours.

### 5. The reaction can be quenched by incubating for 15min at RT with 25 - 60mM of Tris

Add right quantity of 1M Tris.HCl, pH 7.5 (or 1M glycine or lysine).

### 6. The un-reacted reagents and by-products of reaction can be removed by dialysis or other desalting means, while changing buffer to one suitable to storage or further use.

## Protocol<sup>F</sup>: Irreversible immobilisation of antibodies onto an affinity column

This standard protocol describes the coupling attachment of IgG onto a protein A agarose gel using DSS. It should be optimized to each application (volume, type of Ig and gel...). For applications from 1 to 10 mg of IgG, 1 ml of gel is usually needed (pipette a 2ml of 50% suspension). Incubations and washes are performed in batch, with 5 ml buffer per ml of gel.

- 1- Regenerate if needed the agarose protein A gel. Wash well with PBS (150mM NaCl, 20mM phosphate, pH7.4)
- 2- Incubate the antibody in appropriate quantity onto Protein A, during 30 min at room temperature under constant and gentle agitation.  
Uptima recommends a IgG amount equal to the IgG binding capacity of the gel, but lower quantity may be applied depending on the antibody availability and further application requirements
- 3- Wash well with PBS. Absorbance at 280nm should be less than 0.05.  
Rem: the unbound antibody can be recovered in washes and quantified by a protein assay (BC Assay #UP40840A) to determine the loading capacity of the gel, by difference with initial added IgG.
- 4- Prepare a DSS solution at 20 mM in anhydrous DMSO. This solution should be used immediately. The BS3 is dissolved at 20 mM in aqueous buffer.
- 5- Add very slowly 175µl of DSS solution or BS3 solution per ml of gel in 2ml of PBS while stirring. This is advantageous done in 2 or 3 steps separated by 5 min intervals. Incubate for 1H under constant agitation at room temperature.
- 6- Wash the gel with PBS (at least 3 washes of 5 ml per ml of gel during 5minutes)
- 7- Incubate the gel with a saturating agent, 5 ml/ml of gel, during 1H at room temperature. Usually, BSA (#UP900100), normal serum, or SeaBlock (#UP40301) are used, diluted at 5% in PBS.

## References:

- Hermanson, Greg T, "Bioconjugate Techniques", Academic Press, Inc., San Diego, CA, 1996.
- Wong, S.S. (1993) CRC Chemistry of Protein Conjugation and Crosslinking. CRC Press, Boca Raton, Florida.
- Kotite, N.J., Staros, J.V., Cunningham, L.W. (1984). Biochemistry, 23, 3099-3104.
- Dihazi, G.H., Sinz, A. (2003) Rapid Commun. Mass Spectrom. 17, 2005-2014.
- **Loster K, et al**, Chemical cross-linking leads to two high molecular mass aggregates of rat alpha 1 beta 1 integrin differing in their conformation but not in their composition, FEBS Lett., 1995, **373**, 234-238; DSS
  - **Jordan J.A., et al**, Targeting of mouse erythrocytes by band 3 crosslinkers; Biochim.Biophys.Acta, 1291, 27-34 (1996)
  - **Staros J.V.**; N-hydroxysuccinimide active esters: bis(N-hydroxysuccinimide) esters of two dicarboxylic acids are hydrophilic, membrane-impermeant, protein crosslinkers; Biochem. 1982, **21**, 3950-3955; DSS
  - **Waugh, S.M., et al.**, P.F. Isolation of a proteolytically derived domain of the insulin receptor containing the major site of cross-linking/binding. Biochemistry 1989, **28**, 3448-3455; EGS

FT-54940A

### Related products :

See FT-[B4ZBV1](#) for longer PEG products (paucidisperse) and **different succinimide esters (SVA, SCM, SS, SG, GAS, SAS) Mono(Lactosylamido) Mono(Succinimidyl)Suberate #IU7400.** <sup>[L]</sup> MW:594.57; Useful in drug delivery and transport studies.

-Other crosslinkers:

- Heterobifunctional crosslinkers: NHS-MAL reagents, i.e. [NHS-PEO-MAL AL6581](#) and SMCC [17412A](#)
- Homobifunctional crosslinkers: NHS-NHS reagents, i.e. [NHS-PEO-NHS BH8811](#) and DSS [54940A](#)
- Homobifunctional crosslinkers: MAL-MAL reagents, i.e. [MAL-PEO-MAL L7736A](#) and BMOE [L7730A](#)
- PEO Linkers & modifiers: MAL-COOH [AZ4170](#) and BMPA [43064A](#);  
NHS-PEG-COOH [AN1280](#); mPEG-NHS [DZ3531](#) and others (-SH, -OH,...)
- PhotoActivable (PA) crosslinkers: SH and PA reactive i.e. SCBP #[BI1361](#),...• SMCC-hydrazide #[BI1281](#)
- Hydrazone chemistry: [Conjugation kit #BL1501](#) and crosslinkers (SANH #[BL9270](#), MHPH #[BL9401](#) SH-reactive)

## Other Information

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

For any information, please ask : Uptima / Interchim; Hotline : +33(0)4 70 03 73 06  
[Uptima@interchim.com](mailto:Uptima@interchim.com) ; <http://www.interchim.com>

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