

CBDPS-H8/D8**Product Information****CBDPS-H8/D8**

CyanurBiotinDimercaptoPropionylSuccinimide

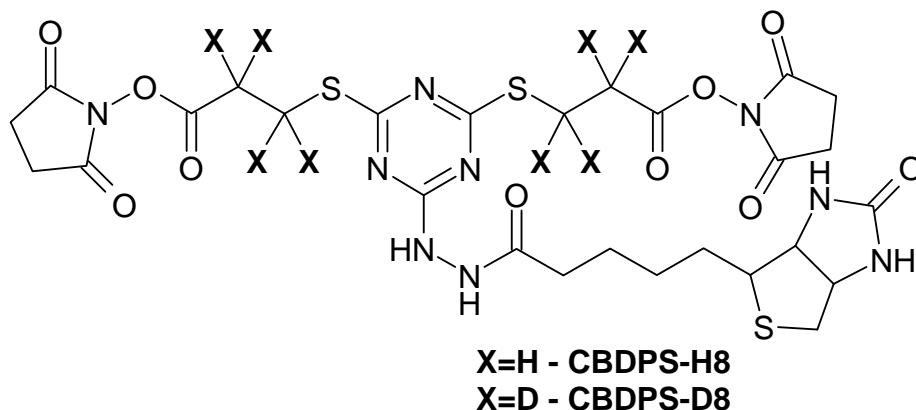
12 x 0.37 mg of 1:1 molar ratio mixture of

CBDPS-H8 and CBDPS-D8

Cat. Number: 014S

Formula: C₂₇H₃₃N₉O₁₀S₃ / C₂₇D₈H₂₅N₉O₁₀S₃

Molecular Weight: 739 / 747

**Features:**

Isotopically-coded.

Biotinylated.

CID-cleavable.

Isotopic MS/MS signatures for dead-end and inter-peptide crosslinks.

CBDPS-H8/D8 is a membrane-permeable, homobifunctional, isotopically-coded, affinity-tagged, CID-cleavable crosslinker CyanurBiotinDimercaptoPropionylSuccinimide. Light (H8) and heavy (D8) forms of the reagent differ by 8 deuterium atoms in heavy form instead of 8 hydrogen atoms of light form, and otherwise are chemically identical. Isotopic coding enables univocal detection of the crosslinked products in mass spectra. Reaction products of CBDPS-H8/D8 will manifest in mass spectra as doublets of peaks of equal intensity corresponding to light (H8) and heavy (D8) forms of the reagent separated by 8.05016 Da divided by charge state (8.05 for +1, 4.03 for +2, 2.68 for +3 etc.).

CBDPS-H8/D8 is biotinylated and therefore CBDPS crosslinks are affinity purifiable with immobilized avidin or streptavidin. Affinity enrichment of the crosslinks allows to eliminate interfering free peptides from the analysis and to enhance detection of the crosslinks.

CBDPS-H8/D8 is CID cleavable. In combination with isotopic coding it creates specific signatures for the cleavage products of the CBDPS crosslinks in MS/MS spectra (Ref. 1).

N-HydroxySuccinimide (NHS) esters react mainly with primary amino groups (-NH₂) in pH 7-9 buffers to form stable amide bonds. Therefore, amine-containing buffers (Tris, Glycine, ammonium salts, etc.) should be avoided for crosslinking reaction. CBDPS is water-insoluble and stock solutions should be prepared in an organic solvent such as DMSO or DMF and then added to the aqueous reaction mixture. To make 10 mM stock solution of the CBDPS-H8/D8, add 50 μl DMSO to the pre-weigh tube containing 0.37 mg (0.5 μmol) of the reagent.

To calculate masses of peptide crosslinks use following formulas:

$$[M_{12}+H]^+ = [M_1+H]^+ + [M_2+H]^+ + 508.08899$$

$$[M_1OH+H]^+ = [M_1+H]^+ + 527.10738$$

$$[M_{1i}+H]^+ = [M_1+H]^+ + 509.09682$$

$$[M_1NH_2+H]^+ = [M_1+H]^+ + 526.12336$$

, where M₁, M₂ - masses of free peptides; M₁₂ – mass of inter-peptide crosslink; M₁OH – mass of dead-end crosslink; M₁i – mass of intra-peptide crosslink; M₁NH₂ – mass of dead-end amide (if reaction was quenched with ammonium salts).

MS-Bridge (<http://prospector.ucsf.edu>) bridge elemental composition: C₁₉ H₂₃ N₇ O₄ S₃; modification elemental composition for –OH dead-ends: C₁₉ H₂₅ N₇ O₅ S₃; modification elemental composition for –NH₂ dead-ends: C₁₉ H₂₅ N₈ O₄ S₃.

Typical MALDI mass spectrum of the test reaction with FLAG (DYKDDDDK) peptide is shown in Figure 1. Masses of the reaction products for the light (H8) form of the reagent are: 1013 – free FLAG peptide; 1522 – intra-peptide crosslink; 1540 – dead-end crosslink; 2534 – inter-peptide crosslink.

CBDPS-H8/D8 crosslinks can be cleaved by CID (Figure 2). Cleaved moieties of the crosslink still isotopically labeled with H₄/D₄ atoms and will manifest in MS/MS spectra as doublets of peaks of equal intensity separated by 4.03 Da divided by charge state. As the cleavage can occur equally at either of two C-S bonds of the crosslinker, it results in two sets of H₄/D₄ doublets separated by 401 Da for each individual peptide constituting inter-peptide crosslink (Figure 2), (Ref. 1).

Cleaved crosslinks masses can be calculated using following formulas:

$$[M_{12}+H]^+ = [M_1cl+H]^+ + [M_2cl+H]^+ + Mcliploss$$

$$[M_1OH+H]^+ = [M_1cl+H]^+ + Mcllohloss$$

$$[M_1i+H]^+ = [M_1icl+H]^+ + Mcliloss$$

$$[M_1cl+H]^+ = [M_1+H]^+ + Mclrest$$

$$[M_1icl+H]^+ = [M_1+H]^+ + Mclirest$$

, where H – mass of proton; M_1 , M_2 – masses of free peptides; M_{12} – mass of inter-peptide crosslink; M_1OH – mass of dead-end cleaved intra-peptide crosslink; Mcliploss, Mcllohloss, Mcliloss – mass additions for cleaved inter-peptide, dead-end and intra-peptide crosslinks, correspondently; Mclrest, Mclirest – mass of cleaved portion of the crosslinking reagent for cleaved inter-peptide or dead-end and intra-peptide crosslinks, correspondently (Table 1).

Table 1. Mass additions for CBDPS crosslinks CID cleavage products.

Reagent	Cleavage	clrest el. comp.	Mclrest	Mclirest	Mcliploss	Mcllohloss	Mcliloss
CBDPS	CID	C16 H21 N7 O3 S3	455.08625	-	-1.00727	72.02058	-
		C3 H2 O1*	54.01002	-	-1.00727	473.09682	-

* - tentatively CID cleavage of proximal C-S bond produces ion of structure $P_1-CO-CH_2-CH_2^+$, where P_1 – peptide moiety.

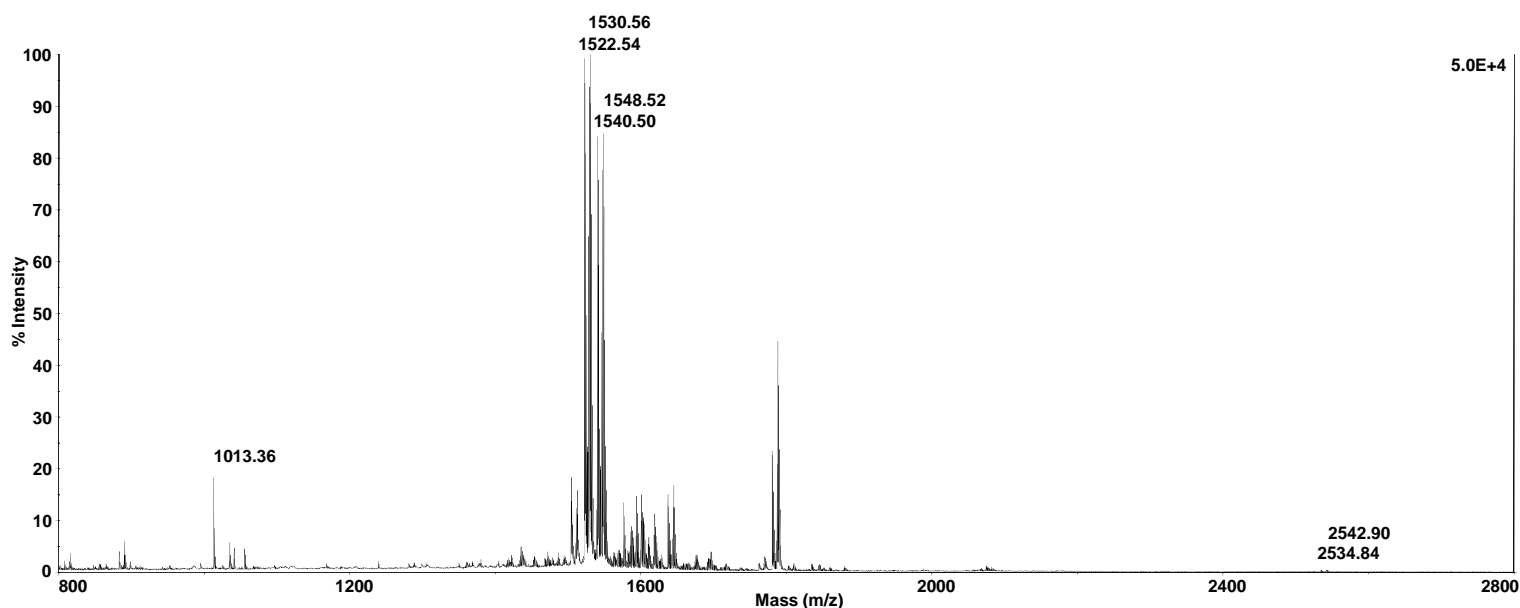


Figure 1. Mass spectrum of reaction products FLAG peptide modified with CBDPS-H8/D8.

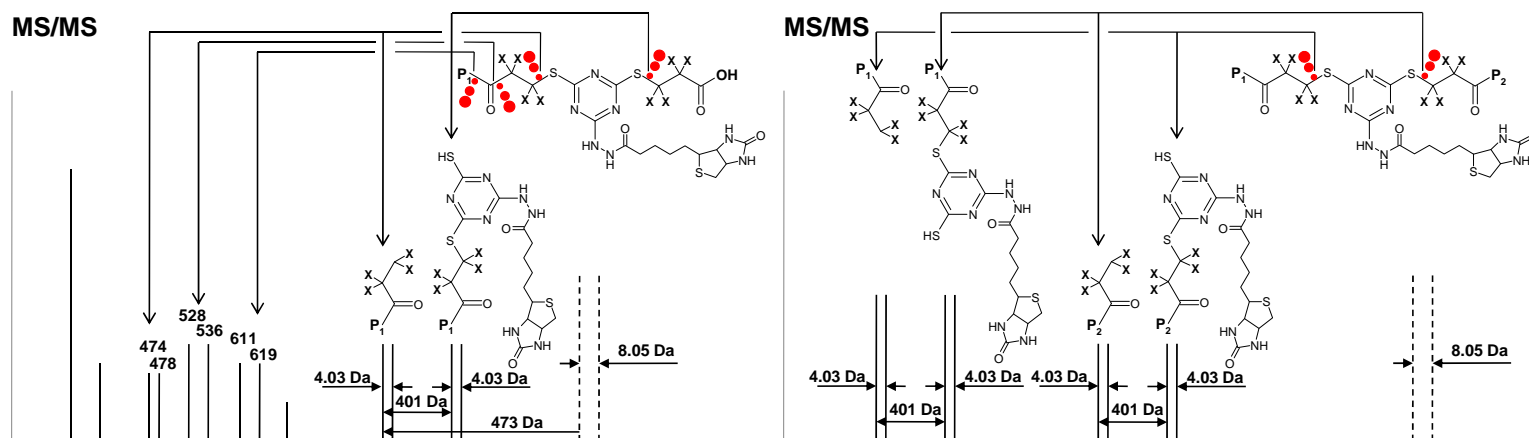


Figure 2. Scheme of CID cleavage of CBDPS-H8/D8 dead-end (left panel) and inter-peptide (right panel) crosslinks.

Material Safety Data information: substance is not fully tested yet.

References:

- Petrotchenko EV, Serpa JJ, Borchers CH. An isotopically-coded CID-cleavable biotinylated crosslinker for structural proteomics. *Mol Cell Proteomics*. 2010 Jul 9. [Epub ahead of print]