Crosslinking reagents masses

Isotopic coding is achieved by using light and heavy forms of the reagents. Reaction products of equimolar mixture of isotopically coded reagents will manifest in mass spectra as doublets of peaks of equal intensity corresponding to light and heavy forms of the reagents separated by specific mass difference according to the number of atoms substituted in the structure of the reagent (Table 1).

Table 1. Isotopic coding mass differences.

Coding	N	Light	Heavy	H-L (+1)	H-L (+2)	H-L (+3)
H12/D12	12	12.08736	24.16260	12.07524	6.03762	4.02508
H8/D8	8	8.05824	16.10840	8.05016	4.02508	2.68339
H6/D6	6	6.04368	12.08130	6.03762	3.01881	2.01254
H4/D4	4	4.02912	8.05420	4.02508	2.01254	1.34169
^{12}C6/^{13}C6	6	71.99670	78.01686	6.02016	3.01008	2.00672

,where H (1.00728 Da), D (2.01355 Da), ^{12}C (11.99945 Da), ^{13}C (13.00281 Da), (MS-Isotope, ProteinProspector).

Crosslinking product masses for the light form of the reagents can be calculated using following formulas:
$[M_{12}+H]^+ = [M_1+H]^+ + [M_2+H]^+ + Mip$
$[M_1OH+H]^+ = [M_1+H]^+ + MOH$
$[M_1i+H]^+ = [M_1+H]^+ + Mi$
$[M_1NH_2+H]^+ = [M_1+H]^+ + MNH_2$
, 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 crosslink; M_1i – mass of intra-peptide crosslink; M_1NH_2 – mass of dead-end amide crosslink (if reaction was quenched with ammonium salts); Mip, MOH, Mi – mass additions for inter-peptide, dead-end and intra-peptide crosslinks, correspondently (Table 2).

Table 2. Mass additions for crosslinking reaction products.

CL	CLbridge el.comp.	MS-Bridge input	Mip	MOH	Mi	MNH_2
DSS	C8 H12 O2	C8 H10 O2	137.06025	156.07864	138.06808	155.09462
DTSP	C6 H8 O2 S2	C6 H6 O2 S2	172.97310	191.99149	173.98093	191.00747
EGS	C10 H12 O6	C10 H10 O6	225.03991	244.05830	226.04774	243.07428
BiPS	C16 H18 N2 O4 S2	C16 H16 N2 O4 S2	363.04732	382.06571	364.05515	381.08169
DNBDPS	C12 H10 N2 O6 S2	C12 H8 N2 O6 S2	338.97455	357.99294	339.98238	357.00892
TEABS	C28 H41 N5 O11 S1	C28 H39 N5 O11 S1	652.22885	671.24724	653.23668	670.26322
DSG	C5 H6 O2	C5 H4 O2	95.01276	114.03115	96.02059	113.04713
DSA	C6 H8 O2	C6 H6 O2	109.02841	128.04680	110.03624	127.06278
CBDPS	C19 H25 N7 O4 S3	C19 H23 N7 O4 S3	508.08899	527.10738	509.09682	526.12336

In case of crosslinks undergoing cleavage, cleaved crosslinks masses can be calculated using following formulas:
$[M_{12}+H]^+ = [M_1cl+H]^+ + [M_2cl+H]^+ + Mcliploss$
$[M_1OH+H]^+ = [M_1cl+H]^+ + Mclohloss$
$[M_1i+H]^+ = [M_1icl+H]^+ + Mcliloss$
$[M_1cl+H]^+ = [M_1+H]^+ + Mclrest$
$[M_1icl+H]^+ = [M_1+H]^+ + Mclirest$
, 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 crosslink; M_1i – mass of intra-peptide crosslink; M_1cl – mass of cleaved dead-end or inter-peptide crosslink; M_1icl – mass of cleaved intra-peptide crosslink; Mcliploss, Mclohloss, 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 3).

Table 3. Mass additions for crosslinks cleavage products.

Reagent	Cleavable	clrest el. comp.	Mclrest	Mclirest	Mcliploss	Mclohloss	Mcliloss
DTSP	DTT	C3 H4 O1 S1	87.99829	175.99657	-3.02349	103.99320	-2.01566
	CID	C3 H4 O1 S2	119.96981	-	-1.00727	72.02058	-
		C3 H2 O1*	54.01002	-	-1.00727	137.98037	-
EGS	NH_4OH	C4 H2 O2	82.00548	164.01096	61.02895	162.05282	62.03678
BiPS	hν	C13 H14 N2 O3 S	278.07251	173.98091	-3.02349	103.99320	190.07422
		C3 H4 O1 S1	87.99829	173.98091	-3.02349	294.06743	190.07422
DNBDPS	DTT, 100oC	C3 H4 O1 S1	87.99829	175.99657	162.97852	269.99465	163.98581
TEABS	NH4OH	C4 H2 O2	82.00548	164.01096	488.21844	589.24121	489.22571
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 C-S bond produces ion of structure P_1-CO-CH_2-CH_2^+, where P_1 – peptide moiety.

When crosslinking reaction is repeated using different crosslinking reagents, inter-relations between crosslinks masses can be calculated using following formula:

$[M_{12}CL1+H]^+ = [M_{12}CL2+H]^+ + MirCL1CL2$

, where H – mass of proton, $M_{12}CL1$, $M_{12}CL2$ – masses of the same crosslinks obtained using reagents CL1 and CL2, correspondently; MirCL1CL2 – mass difference for reagents CL1 and CL2 (Table 4).

Table 4. Mass relationships between crosslinking reagents.

CL1\CL2		DSS	DTSP	EGS	BiPS	DNBDPS	TEABS	DSG	DSA	CBDPS
		137.06025	172.97310	225.03991	363.04732	338.97455	652.22885	95.01276	109.02841	508.08899
DSS	137.06025	0.00000	-35.91285	-87.97966	-225.98707	-201.91430	-515.16860	42.04749	28.03184	-371.02874
DTSP	172.97310	35.91285	0.00000	-52.06681	-190.07422	-166.00145	-479.25575	77.96034	63.94469	-335.11589
EGS	225.03991	87.97966	52.06681	0.00000	-138.00741	-113.93464	-427.18894	130.02715	116.01150	-283.04908
BiPS	363.04732	225.98707	190.07422	138.00741	0.00000	24.07277	-289.18153	268.03456	254.01891	-145.04167
DNBDPS	338.97455	201.91430	166.00145	113.93464	-24.07277	0.00000	-313.25430	243.96179	229.94614	-169.11444
TEABS	652.22885	515.16860	479.25575	427.18894	289.18153	313.25430	0.00000	557.21609	543.20044	144.13986
DSG	95.01276	-42.04749	-77.96034	-130.02715	-268.03456	-243.96179	-557.21609	0.00000	-14.01565	-413.07623
DSA	109.02841	-28.03184	-63.94469	-116.01150	-254.01891	-229.94614	-543.20044	0.00000	0.00000	-399.06058
CBDPS	508.08899	371.02874	335.11589	283.04908	145.04167	169.11444	-144.13986	399.06058	399.06058	0.00000