Thermo Scientific
Crosslinking Technical Handbook

easy molecular bonding

crosslinking technology

Reactivity chemistries, applications and structure references

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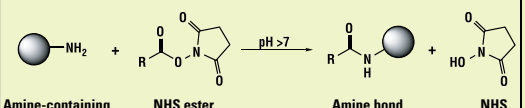
what is crosslinking?

Crosslinking is the process of chemically joining two or more molecules by a covalent bond. Crosslinking reagents contain reactive ends to specific functional groups (primary amines, sulfhydryls, etc.) on proteins or other molecules. The availability of several chemical groups in proteins and peptides make them targets for conjugation and for study using crosslinking methods. Crosslinkers also are commonly used to modify nucleic acids, drugs and solid surfaces. The same chemistry is applied to amino acid and nucleic acid surface modification and labeling. This area of chemistry is known as bioconjugation and includes crosslinking, immobilization, surface modification and labeling of biomolecules. Crosslinking and modification reagents can be described by their chemical reactivity (page 2), molecular properties (page 10) or by their applications (page 14).

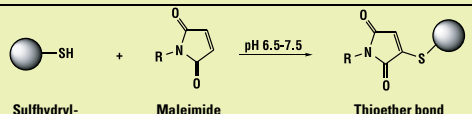
Select the Right Chemistry

First, select a crosslinker with the functional groups to bind your biomolecules of interest.

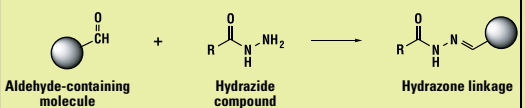
NHS ester reaction



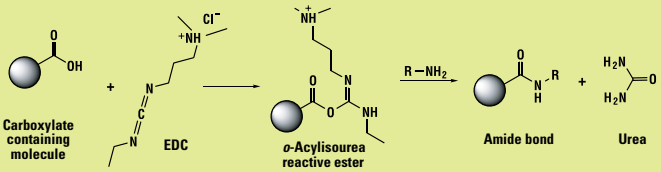
Maleimide reaction



Hydrazide reaction

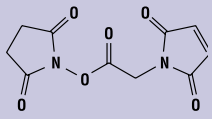
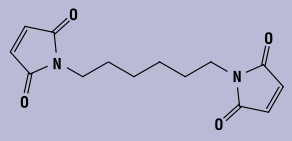
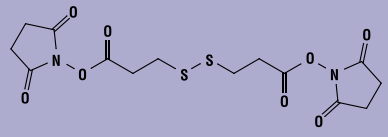
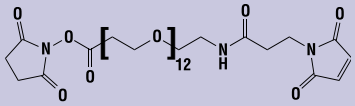
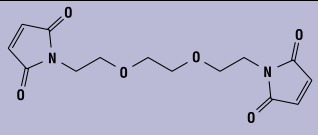


EDC coupling reaction



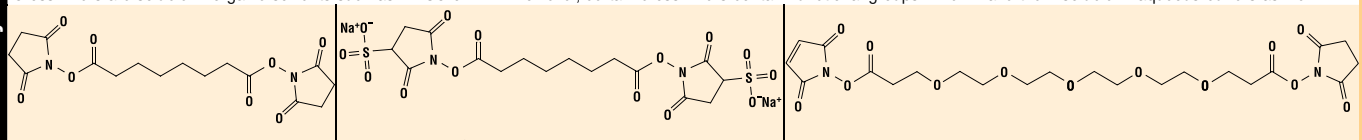
Space Arm Composition

Second, choose which linker characteristics are important for your application.

Length	Composition	Cleavability
 AMAS 4.4Å	 BMH with hydrocarbon spacer	 DSP with disulfide linker for cleavage
 $SM(PEG)_{12}$ 53.4Å	 $BM(PEG)_2$ with polyethylene glycol spacer	 DSS with non-cleaveable spacer arm

Solubility

Crosslinkers are soluble in organic solvents such as DMSO or DMF. However, certain crosslinkers contain functional groups which make them soluble in aqueous buffers as well.



DSS – soluble in organic solvents | BS^3 – soluble in aqueous buffers | $BM(PEG)_5$ – soluble in aqueous buffers

Package Size

Select a package size based on the scale of your reaction. Our crosslinkers are available from mg to kg quantities.

Single-Use	Milligram	Gram	Large/Custom
 Single-use tubes with 2mg crosslinker/vial	 Milligram quantities of crosslinker	 Gram quantities of crosslinker	 Large volume/custom packages

bioconjugation
the core tools

The most important property of a crosslinker is its reactive chemical group. The reactive group establishes the method and mechanism for chemical modification. Crosslinkers contain at least two reactive groups, which target common functional groups found in biomolecules such as proteins and nucleic acids (Figure 1). Protein modification reagents like PEGylation or biotinylation reagents have a reactive group at one terminus and a chemical moiety at the other end (PEG chain or biotin group, respectively). The functional groups that are commonly targeted for bioconjugation include primary amines, sulfhydryls, carbonyls, carbohydrates and carboxylic acids (Table 1). Coupling can also be nonselective using photo-reactive groups.

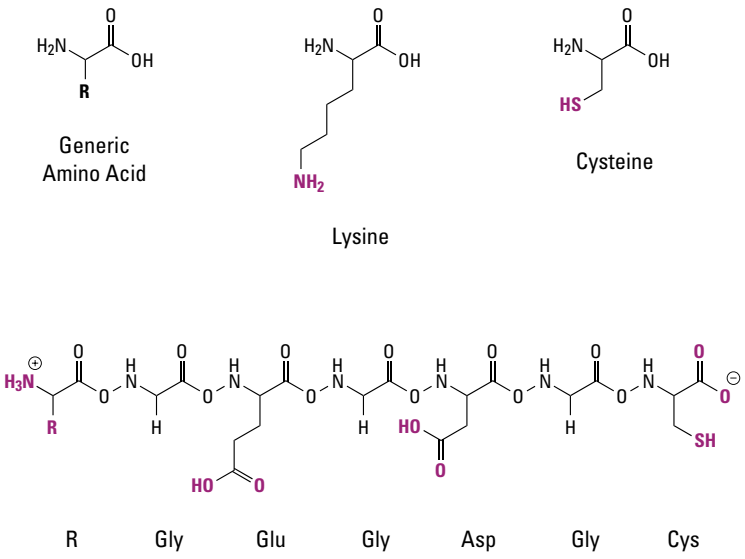


Figure 1. Common amino acid functional groups targeted for bioconjugation.

Table 1. Popular crosslinker reactive groups for protein conjugation.

Reactivity class	Target functional group	Reactive chemical group
Amine-reactive	-NH_2	NHS ester Imidoester Pentafluorophenyl ester Hydroxymethyl phosphine
Carboxyl-to-amine reactive	-COOH	Carbodiimide (e.g., EDC)
Sulfhydryl-reactive	-SH	Maleimide Haloacetyl (Bromo- or Iodo-) Pyridyldisulfide Thiosulfonate Vinylsulfone
Aldehyde-reactive i.e., oxidized sugars (carbonyls)	-CHO	Hydrazide Alkoxyamine
Photo-reactive i.e., nonselective, random insertion	random	Diazirine Aryl azide
Hydroxyl (nonaqueous)-reactive	-OH	Isocyanate
Azide-reactive	-N_3	Phosphine

Amine-reactive chemical groups

Primary amines (–NH_2) exists at the N-terminus of each polypeptide chain (called the alpha-amine) and in the side chain of lysine (Lys, K) residues (called the epsilon-amine). Because of its positive charge at physiologic conditions, primary amines are usually outward facing (i.e., on the outer surface) of proteins, making them more accessible for conjugation without denaturing protein structure. A number of reactive chemical groups target primary amines (Figure 2), but the most commonly used groups are *N*-hydroxysuccinimide esters (NHS esters) and imidoesters.

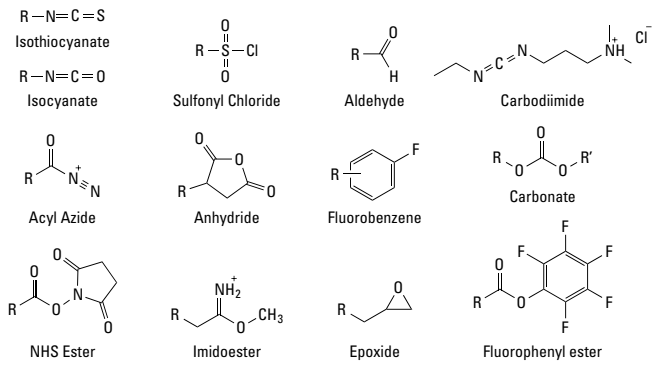
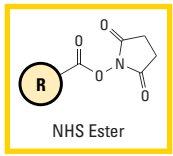


Figure 2. Reactive chemical groups which target primary amines.

• *N*-hydroxysuccinimide esters (NHS esters)



NHS esters are reactive groups formed by EDC activation of carboxylate molecules. NHS ester-activated crosslinkers and labeling compounds react with primary amines in slightly alkaline conditions to yield stable amide bonds. The reaction releases N-hydroxysuccinimide (MW 115g/mol), which can be removed easily by dialysis or desalting.

NHS-reactive chemistry

NHS ester crosslinking reactions are most commonly performed in phosphate, carbonate-bicarbonate, HEPES or borate buffers at pH 7.2-8.5 for 30 minutes to four hours at room temperature or 4°C. Primary amine buffers such as Tris (TBS) are not compatible, because they compete for reaction. However in some procedures, it is useful to add Tris or glycine buffer at the end of a conjugation procedure to stop the reaction.

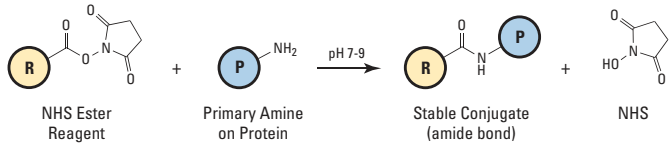
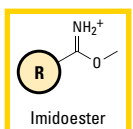


Figure 3. NHS ester reaction scheme for chemical conjugation to a primary amine. (R) represents a labeling reagent or one end of a crosslinker having the NHS ester reactive group; (P) represents a protein or other molecule that contains the target functional group (i.e., primary amine).

Hydrolysis of the NHS ester competes with the primary amine reaction. The rate of hydrolysis increases with buffer pH and contributes to less efficient crosslinking in less concentrated protein solutions. The half-life of hydrolysis for NHS ester compounds is 4 to 5 hours at pH 7.0 and 0°C. This half-life decreases to 10 minutes at pH 8.6 and 4°C. The extent of NHS ester hydrolysis in aqueous solutions free of primary amines can be measured at 260 to 280nm, because the NHS byproduct absorbs in that range.

Sulfo-NHS esters are identical to NHS esters except that they contain a sulfonate (–SO_3) group on the *N*-hydroxysuccinimide ring. This charged group has no effect on the reaction chemistry, but it does tend to increase the water solubility of crosslinkers containing them. In addition, the charged group prevents sulfo-NHS crosslinkers from permeating cell membranes, enabling them to be used for cell surface crosslinking methods.

• Imidoesters



Imidoester crosslinkers react with primary amines to form amidine bonds. To ensure specificity for primary amines, imidoester reactions are best done in amine-free, alkaline conditions (pH 10), such as with borate buffer.

Because the resulting amidine bond is protonated, the crosslink has a positive charge at physiological pH, much like the primary amine which it replaced. For this reason, imidoester crosslinkers have been used to study protein structure and molecular associations in membranes and to immobilize proteins onto solid-phase supports while preserving the isoelectric point (pI) of the native protein. Although imidoesters are still used in certain procedures, the amidine bonds formed are reversible at high pH. Therefore, the more stable and efficient NHS ester crosslinkers have steadily replaced them in most applications.

Imidoester reaction chemistry

Imidoester crosslinkers react rapidly with amines at alkaline pH to form amidine bonds but have short half-lives. As the pH becomes more alkaline, the half-life and reactivity with amines increases, making crosslinking more efficient when performed at pH 10 than at pH 8. Reaction conditions below pH 10 may result in side reactions, although amidine formation is favored between pH 8-10. Studies using monofunctional alkyl imidates reveal that at pH <10, conjugation can form with just one imidoester functional group. An intermediate N-alkyl imidate forms at the lower pH range and will either crosslink to another amine in the immediate vicinity, resulting in N,N'-amidine derivatives, or it will convert to an amidine bond. At higher pH, the amidine is formed directly without formation of an intermediate or side product. Extraneous crosslinking that occurs below pH 10 sometimes interferes with interpretation of results when thiol-cleavable diimidoesters are used.

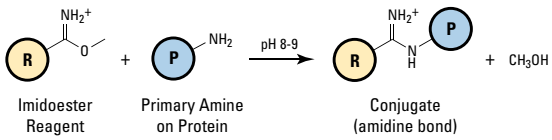
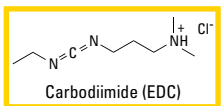


Figure 4. Imidoester reaction scheme for chemical conjugation to a primary amine. (R) represents a labeling reagent or one end of a crosslinker having the imidoester reactive group; (P) represents a protein or other molecule that contains the target functional group (i.e., primary amine, $–NH_2$).

Carboxylic acid-reactive chemical groups

Carboxylic acids (–COOH) exist at the C-terminus of each polypeptide chain and in the side chains of aspartic acid (Asp, D) and glutamic acid (Glu, E). Like primary amines, carboxyls are usually on the surface of protein structure. Carboxylic acids are reactive towards carboiimides.

• Carbodiimides (EDC and DCC)



EDC and other carbodiimides are zero-length crosslinkers. They cause direct conjugation of carboxylates (–COOH) to primary amines ($–NH_2$) without becoming part of the final amide-bond crosslink between target molecules.

Because peptides and proteins contain multiple carboxyls and amines, direct EDC-mediated crosslinking usually causes random polymerization of polypeptides. Nevertheless, this reaction chemistry is used widely in immobilization procedures (e.g., attaching proteins to a carboxylated surface) and in immunogen preparation (e.g., attaching a small peptide to a large carrier protein).

EDC reaction chemistry

EDC reacts with carboxylic acid groups to form an active *O*-acylisourea intermediate that is easily displaced by nucleophilic attack from primary amino groups in the reaction mixture. The primary amine forms an amide bond with the original carboxyl group, and an EDC by-product is released as a soluble urea derivative. The *O*-acylisourea intermediate is unstable in aqueous solutions. Failure to react with an amine results in hydrolysis of the intermediate, regeneration of the carboxyls, and the release of an N-unsubstituted urea.

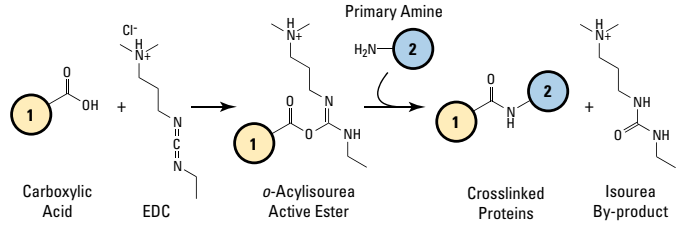


Figure 5. EDC (carbodiimide) crosslinking reaction scheme: Carboxyl-to-amine crosslinking with the popular carbodiimide, EDC. Molecules (1) and (2) can be peptides, proteins or any chemicals that have respective carboxylate and primary amine groups. When they are peptides or proteins, these molecules are tens-to-thousands of times larger than the crosslinker and conjugation arms diagrammed in the reaction.

EDC crosslinking is most efficient in acidic (pH 4.5) conditions and must be performed in buffers devoid of extraneous carboxyls and amines. MES buffer (4-morpholinoethanesulfonic acid) is a suitable carbodiimide reaction buffer. Phosphate buffers and neutral pH (up to 7.2) conditions are compatible with the reaction chemistry, but with lower efficiency. Increasing the amount of EDC in a reaction solution can compensate for the reduced efficiency.

N-hydroxysuccinimide (NHS) or its water-soluble analog (sulfo-NHS) is often included in EDC coupling protocols to improve efficiency or create dry-stable (amine-reactive) intermediates (Figure 6). EDC couples NHS to carboxyls, forming an NHS ester that is considerably more stable than the *O*-acylisourea intermediate while allowing for efficient conjugation to primary amines at physiologic pH.

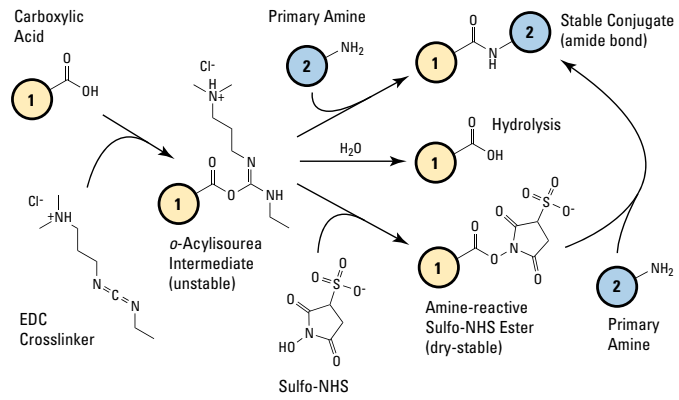


Figure 6. Sulfo-NHS plus EDC (carbodiimide) crosslinking reaction scheme: Carboxyl-to-amine crosslinking using the carbodiimide EDC and Sulfo-NHS. Addition of NHS or Sulfo-NHS to EDC reactions (bottom-most pathway) increases efficiency and enables molecule (1) to be activated for storage and later use.

EDC is also capable of activating phosphate groups in the presence of imidazole for conjugation to primary amines. The method is sometimes used to modify, label, crosslink or immobilize oligonucleotides through their 5' phosphate groups.

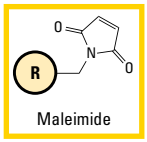
DCC reaction chemistry and applications

DCC (dicyclohexyl carbodiimide) crosslinks carboxylic acids to primary amines in the same manner as EDC. However, because DCC is not aqueous-soluble, it is primarily used in manufacturing and organic synthesis applications rather than in the typical protein research biology lab. For example, most commercially available, ready-to-use NHS ester crosslinkers and labeling reagents are manufactured using DCC. Because water is excluded, the resulting NHS ester can be prepared and stabilized as a dried powder without appreciable hydrolysis. DCC is also commonly used in commercial peptide synthesis operations.

Sulfhydryl-reactive chemical groups

Sulfhydryls (–SH) exist in the side chain of cysteine (Cys, C). Often, as part of a protein's secondary or tertiary structure, cysteines are joined together between their side chains via disulfide bonds (–S–S–). These must be reduced to sulfhydryls to make them available for crosslinking by most types of reactive groups. Sulfhydryls are reactive towards maleimides, haloacetyls and pyridyl disulfides.

• Maleimides



Maleimide-activated crosslinkers and labeling reagents react specifically with sulfhydryl groups (–SH) at near neutral conditions (pH 6.5-7.5) to form stable thioether linkages. Disulfide bonds in protein structures must be reduced to free thiols (sulfhydryls) to react with maleimide reagents. Extraneous thiols (e.g., from most reducing agents) must be excluded from maleimide reaction buffers because they will compete for coupling sites.

Short homobifunctional maleimide crosslinkers enable disulfide bridges in protein structures to be converted to permanent, irreducible linkages between cysteines. More commonly, the maleimide chemistry is used in combination with amine-reactive, NHS ester chemistry in the form of heterobifunctional crosslinkers that enable controlled, two-step conjugation of purified peptides and/or proteins.

Maleimide reaction chemistry

The maleimide group reacts specifically with sulfhydryl groups when the pH of the reaction mixture is between pH 6.5 and 7.5, resulting in the formation of a stable thioether linkage that is not reversible (Figure 7). In more alkaline conditions (pH >8.5), the reaction favors primary amines and also increases the rate of hydrolysis of the maleimide group to a non-reactive maleamic acid. Maleimides do not react with tyrosines, histidines or methionines.

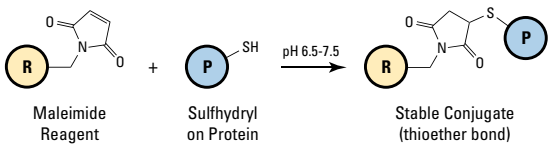
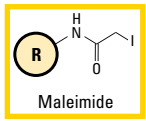


Figure 7. Maleimide reaction scheme for chemical conjugation to a sulfhydryl. (R) represents a labeling reagent or one end of a crosslinker having the maleimide reactive group; (P) represents a protein or other molecule that contains the target functional group (i.e., sulfhydryl, –SH).

Thiol-containing compounds, such as dithiothreitol (DTT) and beta-mercaptoethanol (BME), must be excluded from reaction buffers used with maleimides because they will compete for coupling sites. For example, if DTT were used to reduce disulfides in a protein to make sulfhydryl groups available for conjugation, the DTT would have to be thoroughly removed using a desalting column before initiating the maleimide reaction. Interestingly, the disulfide-reducing agent TCEP does not contain thiols and does have to be removed before reaction using maleimide reagents.

Excess maleimides can be quenched at the end of a reaction by adding free thiols. EDTA can be included in the coupling buffer to chelate stray divalent metals that otherwise promote oxidation of sulfhydryls (non-reactive).

• Haloacetyls



Most haloacetyl crosslinkers contain an iodoacetyl or a bromoacetyl group. Haloacetyls react with sulfhydryl groups at physiologic to alkaline conditions (pH 7.2-9), resulting in stable thioether linkages. To limit free iodine generation, which has the potential to react with tyrosine, histidine and tryptophan residues, it is best to perform iodoacetyl reactions in the dark.

Haloacetyl reaction chemistry

Haloacetyls react with sulfhydryl groups at physiologic pH. The reaction of the iodoacetyl group proceeds by nucleophilic substitution of iodine with a sulfur atom from a sulfhydryl group, resulting in a stable thioether linkage (Figure 8). Using a slight excess of the iodoacetyl group over the number of sulfhydryl groups at pH 8.3 ensures sulfhydryl selectivity. In the absence of free sulfhydryls, or if a large excess of iodoacetyl group is used, the iodoacetyl group can react with other amino acids. Imidazoles can react with iodoacetyl groups at pH 6.9-7.0, but the incubation must proceed for longer than one week.

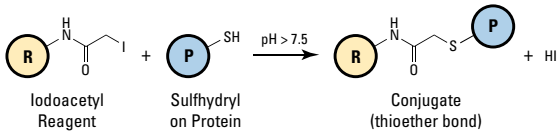
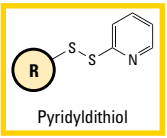


Figure 8. Iodoacetyl reaction scheme for chemical conjugation to a sulfhydryl. (R) represents a labeling reagent or one end of a crosslinker having the iodoacetyl or bromoacetyl reactive group; (P) represents a protein or other molecule that contains the target functional group (i.e., sulfhydryl, –SH).

Histidyl side chains and amino groups react in the unprotonated form with iodoacetyl groups above pH 5 and pH 7, respectively. To limit free iodine generation, which has the potential to react with tyrosine, histidine and tryptophan residues, perform iodoacetyl reactions and preparations in the dark. Avoid exposure of iodoacetyl compounds to reducing agents.

• Pyridyl disulfides



Pyridyl disulfides react with sulfhydryl groups over a broad pH range to form disulfide bonds. As such, conjugates prepared using these crosslinkers are cleavable with typical disulfide reducing agents, such as dithiothreitol (DTT).

Pyridyl disulfide reaction chemistry

Pyridyl disulfides react with sulfhydryl groups over a broad pH range (the optimum is pH 4-5) to form disulfide bonds. During the reaction, a disulfide exchange occurs between the molecule's –SH group and the reagent's 2-pyridyldithiol group. As a result, pyridine-2-thione is released and can be measured spectrophotometrically (A_{max} = 343nm) to monitor the progress of the reaction. These reagents can be used as crosslinkers and to introduce sulfhydryl groups into proteins. The disulfide exchange can be performed at physiologic pH, although the reaction rate is slower than in acidic conditions.

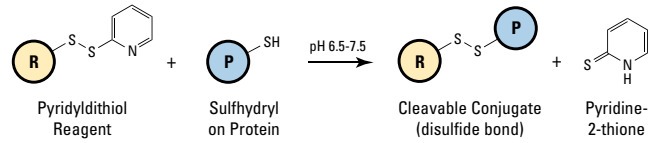


Figure 9. Pyridyldithiol reaction scheme for cleavable (reversible) chemical conjugation to a sulfhydryl. (R) represents a labeling reagent or one end of a crosslinker having the pyridyl disulfide reactive group; (P) represents a protein or other molecule that contains the target functional group (i.e., sulfhydryl, –SH).

Carbonyl-reactive chemical groups

Carbonyl (–CHO) groups can be created in glycoproteins by oxidizing the polysaccharide post-translational modifications with sodium *meta*-periodate. Hydrazide and alkoxyamine reactive groups target aldehydes. These aldehydes also react with primary amines to form Schiff bases which can be further reduced to form a covalent bond (reductive amination).

Carbohydrate modification is particularly useful for creating target sites for conjugation on polyclonal antibodies because the polysaccharides are located in the Fc region. This results in labeling or crosslinking sites located away from antigen binding sites, ensuring that antibody function will not be adversely affected by the conjugation procedure.

• Carbonyls (aldehydes) as crosslinking targets

Aldehydes (RCHO) and ketones (RCOR') are reactive varieties of the more general functional group called carbonyls, which have a carbon-oxygen double bond (C=O). The polarity of this bond (especially in the context of aldehydes) makes the carbon atom electrophilic and reactive to nucleophiles such as primary amines.

Although aldehydes do not naturally occur in proteins or other macromolecules of interest in typical biological samples, they can be created wherever oxidizable sugar groups (also called reducing sugars) exist. Such sugars are common monomer constituents of the polysaccharides or carbohydrates in post-translational glycosylation of many proteins. In addition, the ribose of RNA is a reducing sugar.

Periodic acid (HIO_4) from dissolved sodium periodate ($NaIO_4$) is a well known mild agent for effectively oxidizing vicinal diols in carbohydrate sugars to yield reactive aldehyde groups. The carbon-carbon bond is cleaved between adjacent hydroxyl groups. By altering the amount of periodate used, aldehydes can be produced on a smaller or larger selection of sugar types. For example, treatment of glycoproteins with 1mM periodate usually affects only sialic acid residues, which frequently occur at the ends of polysaccharide chains. At concentrations of 6 to 10mM periodate, other sugar groups in proteins will be affected (Figure 10).

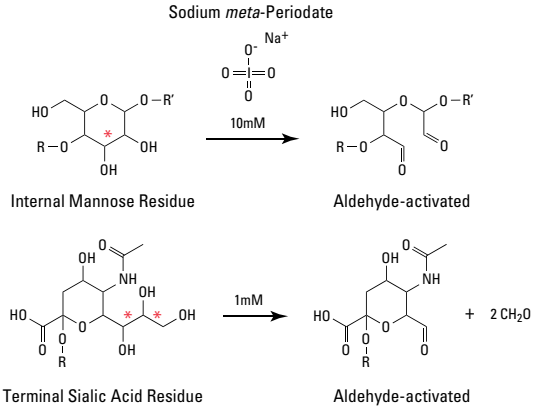
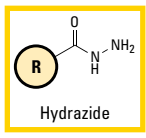


Figure 10. The reaction of sodium periodate with sugar residues yields aldehydes for conjugation reactions. R and R' represent connecting sugar monomers of the polysaccharide. Red asterisks indicate sites of diol cleavage. Sialic acid is also called N-acetyl-D-neuraminic acid.

• Hydrazides



Carbonyls (aldehydes and ketones) can be produced in glycoproteins and other polysaccharide-containing molecules by mild oxidation of certain sugar glycols using sodium *meta*-periodate. Hydrazide-activated crosslinkers and labeling compounds will then conjugate with these carbonyls at pH 5-7, resulting in formation of hydrazone bonds.

Hydrazide chemistry is useful for labeling, immobilizing or conjugating glycoproteins through glycosylation sites, which are often (as with most polyclonal antibodies) located at domains away from the key binding sites whose function one wishes to preserve.

Hydrazide reaction chemistry

Aldehydes created by periodate-oxidation of sugars in biological samples react with hydrazides at pH 5-7 to form hydrazone bonds (Figure 11). Although this bond to a hydrazide group is a type of Schiff base, it is considerably more stable than a Schiff base formed with a simple amine. The hydrazone bond is sufficiently stable for most protein-labeling applications. If desired, however, the double bond can be reduced to a more stable secondary amine bond using sodium cyanoborohydride (see section on reductive amination).

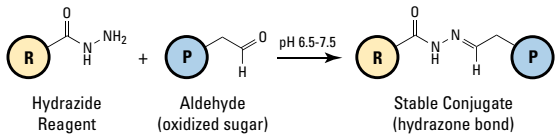
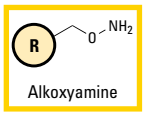


Figure 11. Hydrazide reaction scheme for chemical conjugation to an aldehyde. (R) represents a labeling reagent or one end of a crosslinker having the hydrazide reactive group; (P) represents a glycoprotein or other glycosylated molecule that contains the target functional group (i.e., an aldehyde formed by periodate oxidation of carbohydrate-sugar groups, such as sialic acid).

• Alkoxyamines



Although not currently as popular or common as hydrazide reagents, alkoxyamine compounds conjugate to carbonyls (aldehydes and ketones) in much the same manner as hydrazides.

Alkoxyamine reaction chemistry

Alkoxyamine compounds conjugate to carbonyls to create an oxime linkage. The reaction is similar to hydrazides, and can also use aniline as a catalyst.

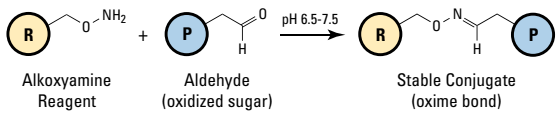


Figure 12. Alkoxyamine reaction scheme for chemical conjugation to an aldehyde. (R) represents a labeling reagent or one end of a crosslinker having the alkoxyamine reactive group; (P) represents a glycoprotein or other glycosylated molecule that contains the target functional group (i.e., an aldehyde formed by periodate oxidation of carbohydrate-sugar groups, such as sialic acid).

• Reductive amination

In reductive amination, the electrophilic carbon atom of an aldehyde attacks the nucleophilic nitrogen of a primary amine to yield a weak bond called a Schiff base. Unlike the bond formed with hydrazide or alkoxyamines, the Schiff base formed with ordinary amines rapidly hydrolyzes (reverses) in aqueous solution and must be reduced to an alkylamine (secondary amine) linkage to stabilize it. Sodium cyanoborohydride ($NaCNBH_3$) is a mild reducing agent that performs this function effectively, without reducing other chemical groups in biological samples (Figure 13). Like Carbodiimide crosslinking chemistry (carboxyl-to-amine), reductive amination (aldehyde-to-amine) is a zero-length crosslinking method.

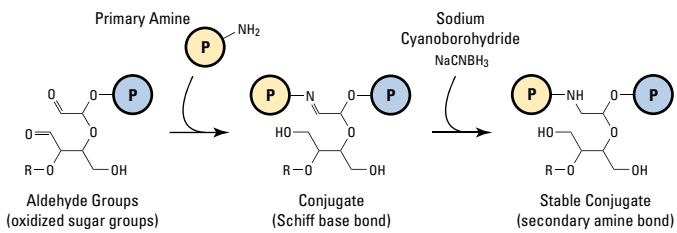


Figure 13. Reductive amination, the conjugation of aldehydes and primary amines. The initial reaction results in a weak, reversible Schiff base linkage. Reduction with sodium cyanoborohydride creates a stable, irreversible secondary amine bond.

Nonspecific-reactive chemical groups

Photo-reactive crosslinkers are widely used for nonspecific bioconjugation. While numerous options exist (see Figure 14), the two most common photo-reactive chemical groups are diazirines and aryl-azides. Photo-reactive groups are activated by UV light and can be used *in vitro* and *in vivo*.

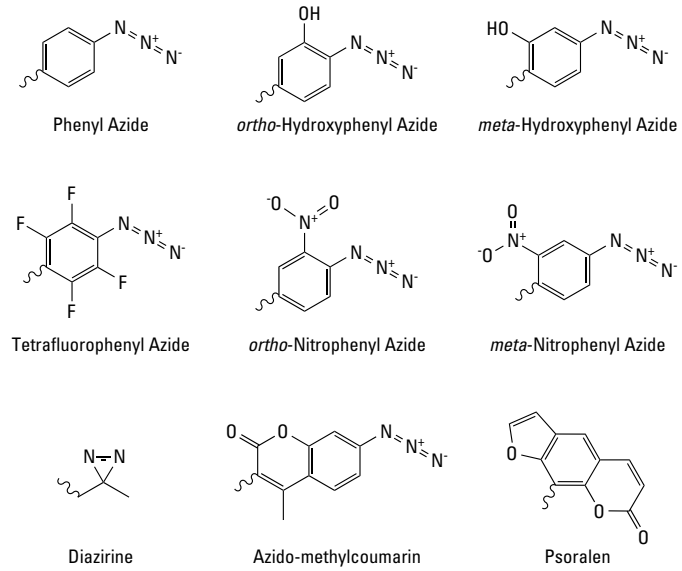


Figure 14. Common photo-reactive chemical groups used for bioconjugation.

• Aryl azides

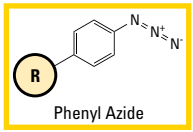


Photo-reactive reagents are chemically inert compounds that become reactive when exposed to ultraviolet or visible light. Historically, aryl azides (also called phenylazides) have been the most popular photo-reactive chemical group used in crosslinking and labeling reagents.

Photo-reactive reagents are most often used as heterobifunctional crosslinkers to capture binding partner interactions. A purified bait protein is labeled with the crosslinker using the amine- or sulfhydryl-reactive end. Then this labeled protein is added to a lysate sample and allowed to bind its interactor. Finally, photo-activation with UV light initiates conjugation via the phenyl azide group.

Aryl azide reaction chemistry

When an aryl azide is exposed to UV light (250 to 350nm), it forms a nitrene group that can initiate addition reactions with double bonds, insertion into C–H and N–H sites, or subsequent ring expansion to react with a nucleophile (e.g., primary amines). The latter reaction path dominates when primary amines are present in the sample.

Thiol-containing reducing agents (e.g., DTT or 2-mercaptoethanol) must be avoided in the sample solution during all steps before and during photo-activation, because they reduce the azide functional group to an amine, preventing photo-activation. Reactions can be performed in a variety of amine-free buffer conditions. If working with heterobifunctional photo-reactive crosslinkers, use buffers compatible with both reactive chemistries involved. Experiments must be performed in subdued light and/or with reaction vessels covered in foil until photo-reaction is intended. Typically, photo-activation is accomplished with a hand-held UV lamp positioned close to the reaction solution and shining directly on it (i.e., not through glass or polypropylene) for several minutes.

Three basic forms of aryl azides exist: simple phenyl azides, hydroxyphenyl azides and nitrophenyl azides. Generally, short-wavelength UV light (e.g., 254nm; 265-275nm) is needed to efficiently activate simple phenyl azides, while long UV light (e.g., 365nm; 300- 460nm) is sufficient for nitrophenyl azides. Because short-wave UV light can be damaging to other molecules, nitrophenyl azides are usually preferable for crosslinking experiments.

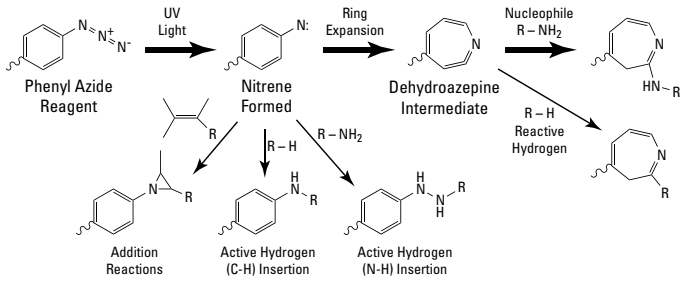
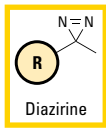


Figure 15. Aryl azide reaction scheme for light-activated photochemical conjugation. Squiggle bonds represent a labeling reagent or one end of a crosslinker having the phenyl azide reactive group; (R) represents a protein or other molecule that contains nucleophilic or active hydrogen groups. Bold arrows indicate the dominant pathway. Halogenated aryl azides react directly (without ring-expansion) from the activated nitrene state.

• Diazirines



Diazirines are a newer class of photo-activatable chemical groups that are being used in crosslinking and labeling reagents. The diazirine (azipentanoate) moiety has better photostability than phenyl azide groups, and it is more easily and efficiently activated with long-wave UV light (330-370nm).

Diazirine reaction chemistry

Photo-activation of diazirine creates reactive carbene intermediates. Such intermediates can form covalent bonds through addition reactions with any amino acid side chain or peptide backbone at distances corresponding to the spacer arm lengths of the particular reagent. Diazirine analogs of amino acids can be incorporated into protein structures by translation, enabling specific recombinant proteins to be activated as the crosslinker.

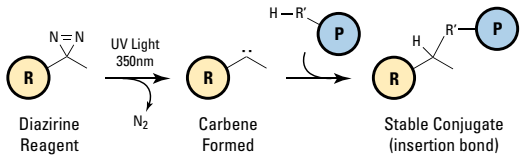
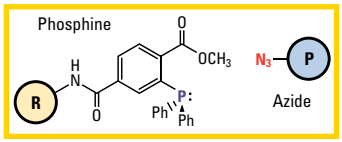


Figure 16. Diazirine reaction scheme for light-activated photochemical conjugation. (R) represent a labeling reagent or one end of a crosslinker having the diazirine reactive group; (P) represents a protein or other molecule that contains nucleophilic or active hydrogen groups (R').

Chemoselective ligation



Chemoselective ligation refers to the use of mutually specific pairs of conjugation reagents. Staudinger ligation reagents are pairs of metabolic or chemical labeling compounds that have azide and phosphine groups, respectively. These reactive groups recognize each other but not any natural or endogenous biomolecules in typical samples. When respective pairs of target biomolecules are labeled with these two groups, they will conjugate to one another with high specificity when combined. This specialized form of crosslinking is most often used for *in vivo* metabolic labeling.

• Chemoselectivity of azide-phosphine reactions

The Staudinger reaction occurs between a methyl ester phosphine (P_3) and an azide (N_3) to produce an aza-ylide intermediate that is trapped to form a stable covalent bond. This crosslinking chemistry, invented in the 1900s by polymer chemist and Nobel Laureate (1953) Hermann Staudinger, has only recently been applied to biological systems as a bioconjugation technique. The chemical biology application is now known as Staudinger ligation.

Unlike typical crosslinking methods used in biological research, this reaction chemistry depends upon a pair of unique reactive groups that are specific to one another and also foreign to biological systems. Because phosphines and azides do not occur in cells, they react only with each other in biological samples, resulting in minimal background and few artifacts. This is the meaning of “chemoselective”.

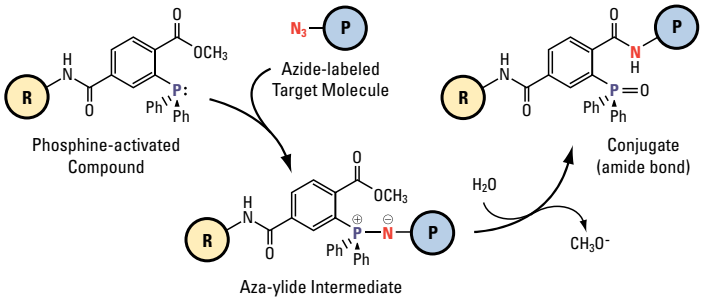
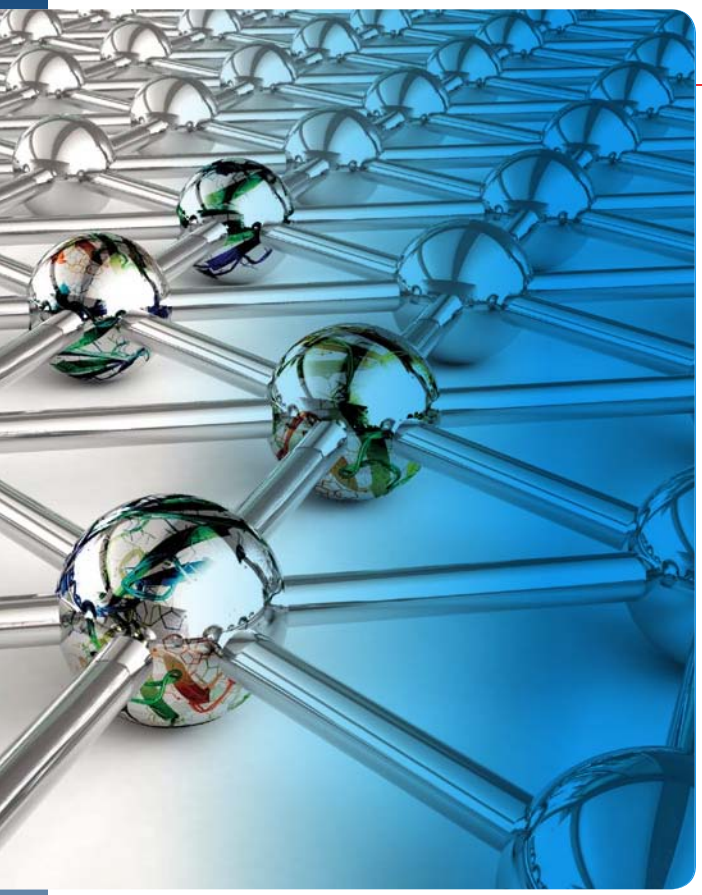


Figure 17. Staudinger ligation reaction scheme (azide-phosphine conjugation). Phosphine-activated proteins or labeling reagents react with azide-labeled target molecules to form aza-ylide intermediates that quickly rearrange in aqueous conditions to form stable amide bonds between reactant molecules.



molecular properties
compatible with various applications

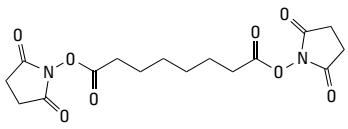
Crosslinkers are selected on the basis of their chemical reactivities and other chemical properties that affect their behavior in different applications. These structural properties are described in Table 2.

Table 2. Molecular properties of crosslinkers and modifiers.

Chemical specificity	The reactive target(s) of the crosslinker's reactive ends. A general consideration is whether the reagent has the same or different reactive groups at either end (termed homobifunctional and heterobifunctional, respectively).
General reaction conditions	The buffer system required to perform bioconjugation. Variables include pH, buffer concentration and protein concentration.
Spacer arm length	The molecular span of a crosslinker (i.e., the distance between conjugated molecules). A related consideration is whether the linkage is cleavable or reversible.
Space arm composition	The chemical groups found within the spacer arm.
Space arm cleavability	The availability of a cleavage site within the spacer arm between the chemical reactive groups.
Chain structure	The presence of a straight or branched chain.
Water solubility and cell membrane permeability	Affect whether a crosslinker or modifier can permeate into cells and/or crosslink hydrophobic proteins within membranes. These properties are determined by the composition of the spacer arm and/or reactive group.

Homobifunctional and heterobifunctional crosslinkers

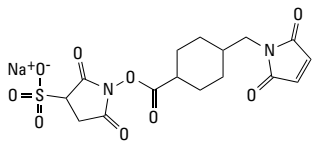
Crosslinkers can be classified as homobifunctional or heterobifunctional. Homobifunctional crosslinkers have identical reactive groups at either end of a spacer arm. Generally, they must be used in one-step reaction procedures to randomly "fix" or polymerize molecules containing like functional groups. For example, adding an amine-to-amine crosslinker to a cell lysate will result in random conjugation of protein subunits, interacting proteins and any other polypeptides whose lysine side chains happen to be near each other in the solution. This is ideal for capturing a "snapshot" of all protein interactions but cannot provide the precision needed for other types of crosslinking applications. For example, when preparing an antibody-enzyme conjugate, the goal is to link one to several enzyme molecules to each molecule of antibody without causing any antibody-to-antibody linkages to form. This is not possible with homobifunctional crosslinkers.



DSS
Disuccinimidyl suberate
MW 368.34
Spacer Arm 11.4Å

Figure 18. Homobifunctional crosslinker example. DSS is a popular, simple crosslinker that has identical amine-reactive NHS ester groups at either end of a short spacer arm. The spacer arm length (11.4 angstroms) is the final maximum molecular distance between conjugated molecules (i.e., nitrogens of the target amines).

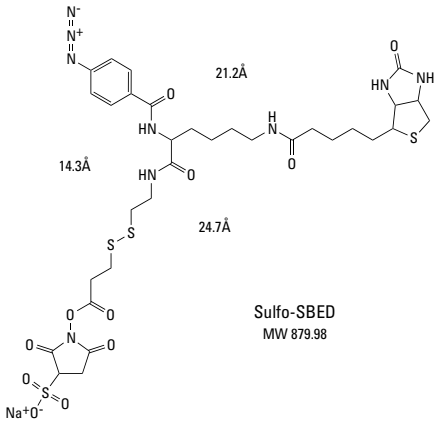
Heterobifunctional crosslinkers possess different reactive groups at either end. These reagents not only allow for single-step conjugation of molecules that have the respective target functional groups, but they also allow for sequential (two-step) conjugations that minimize undesirable polymerization or self-conjugation. In sequential procedures, heterobifunctional reagents are reacted with one protein using the most labile group of the crosslinker first. After removing excess unreacted crosslinker, the modified first protein is added to a solution containing the second protein where reaction through the second reactive group of the crosslinker occurs. The most widely-used heterobifunctional crosslinkers are those having an amine-reactive succinimidyl ester (NHS ester) at one end and a sulfhydryl-reactive group (e.g., maleimide) on the other end. Because the NHS ester group is less stable in aqueous solution, it is usually reacted to one protein first. If the second protein does not have available native sulfhydryl groups, they can be added in a separate prior step using sulfhydryl-addition reagents.



Sulfo-SMCC
Sulfosuccinimidyl 4-(*N*-maleimidomethyl)cyclohexane-1-carboxylate
MW 436.37
Spacer Arm 8.3Å

Figure 19. Heterobifunctional crosslinker example. Sulfo-SMCC is a popular crosslinker that has an amine-reactive sulfo-NHS ester group (left) at one end and a sulfhydryl reactive maleimide group (right) at the opposite end of a cyclohexane spacer arm. This allows for sequential, two-step conjugation procedures.

Certain reagents have three termini, and are referred to as trifunctional crosslinkers or label transfer reagents. These compounds typically possess two chemically reactive functional groups and one label such as a biotin group. A commonly used example of this is Sulfo-SBED.



Sulfo-SBED
MW 879.98

Figure 20. Trifunctional crosslinker example: Sulfo-SBED.

General reaction conditions

In many applications, it is necessary to maintain the native structure of the protein complex, so crosslinking is most often performed using near-physiologic conditions. Optimal crosslinker-to-protein molar ratios for reactions must be determined empirically, although product instructions for individual reagents generally contain guidelines and recommendations for common applications.

Depending on the application, the degree of conjugation is an important factor. For example, when preparing immunogen conjugates, a high degree of conjugation is desired to increase the immunogenicity of the antigen. However, when conjugating to an antibody or an enzyme, a low-to-moderate degree of conjugation may be optimal so that biological activity of the protein is retained.

structural properties

of crosslinkers and modification reagents

The number of functional groups on the protein's surface is also important to consider. If there are numerous target groups, a lower crosslinker-to-protein ratio can be used. For a limited number of potential targets, a higher crosslinker-to-protein ratio may be required. Furthermore, the number of components should be kept low or to a minimum because conjugates consisting of more than two components are difficult to analyze and provide less information on spatial arrangements of protein subunits.

Table 3. Summary of characteristics of crosslinker reactive groups.

Reactive group	Target molecule	Reaction buffer	Optimal reaction pH range
Amine	Aldehydes (oxidized carbohydrates) Carboxylic acid (EDC-modified)	PBS (non-amine) MES	pH 7.2 pH 4.5-7.2
Aryl azide	Unsubstituted aryl azides react primarily with amines	PBS (non-amine)	
Carbodiimide	Carboxylic acids, hydroxyls	MES or PBS	pH 4.5-7.2
Hydrazide	Aldehydes, ketones Carboxylic acid (EDC modified)	0.1M Na-acetate / phosphate Mes/non-amine buffer	pH 5.5-7.5 pH 4.5-7.2 Up to pH 7.5
Imidoester	Amines	PBS, borate, carbonate/ bicarbonate, HEPES	pH 8-9
Iodoacetyl	Sulfhydryls	PBS, borate, carbonate/ bicarbonate, HEPES	pH 7.5-8.5
Isocyanate (PMPI)	Hydroxyls Amines	Nonaqueous	
Maleimide	Sulfhydryls	Thiol-free	pH 7 optimal pH 6.5-7.5
NHS ester	Amines	PBS, borate, carbonate/ bicarbonate, HEPES	pH 7.5 optimal pH 7.2-8.5
Pyridyl disulfide	Sulfhydryls	Thiol-free	pH 7-8
Vinyl sulfone (HBVS)	Sulfhydryls	Thiol-free	pH 8

Spacer arm length

The spacer arm is the chemical chain between two reactive groups. The length of a spacer arm (measured in angstroms) determines how flexible a conjugate will be. Longer spacer arms have greater flexibility and reduced steric hindrance. Longer spacer arms have the caveat of possessing more sites for potential nonspecific binding. Spacer arms can range from zero length to > 100 angstroms (Figure 21).

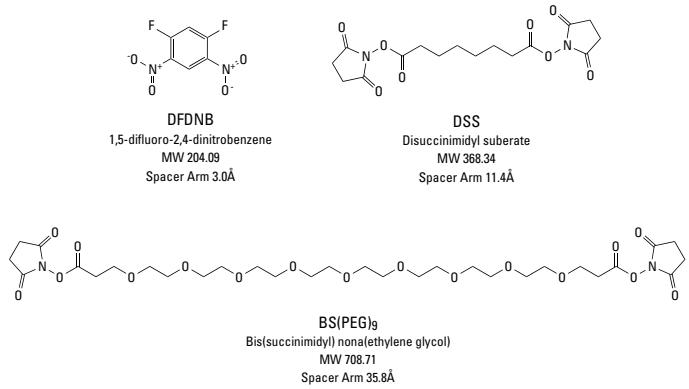


Figure 21. Different length spacer arms.

Spacer arm composition

The molecular composition of a crosslinkers spacer arm can affect solubility and nonspecific binding. Traditional crosslinkers have spacer arms that contain hydrocarbon chains or polyethylene glycol (PEG) chains. Hydrocarbon chains are not water soluble and typically require an organic solvent such as DMSO or DMF for suspension. These crosslinkers are better suited for penetrating the cell membrane and performing intercellular crosslinking because they are hydrophobic and uncharged. If a charged sulfonate group is added to the termini of these crosslinkers, a water soluble analogue is formed. A good example of this is the comparison of DSS with BS^3. DSS is soluble in organic solvents whereas BS^3 is soluble is aqueous buffers. $BS(PEG)_5$ is also water soluble because of its PEG spacer (Figure 22).

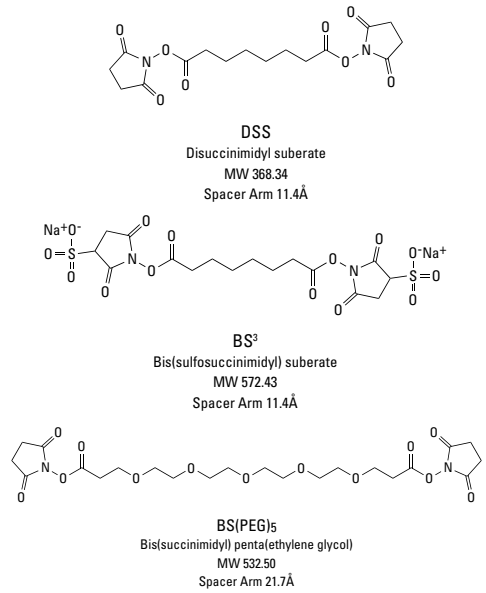


Figure 22. Crosslinked with various spacer arm compositions.

Spacer arm cleavability

Crosslinkers and protein modification reagents form stable, covalent bonds with the proteins they react with. In certain applications, it is desirable to have the ability to break that bond and recover the individual components. Crosslinkers are available with a cleavage site built into the spacer arm. The most commonly used cleavage site is a di-sulfide bridge, which can be readily reduced with the introduction of a common reducing agent such as ß-mercapatoethanol, dithiothreitol or TCEP. An example of this is the crosslinker DTBP (Figure 23).

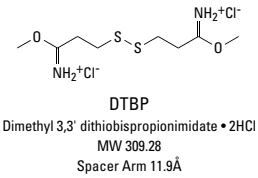


Figure 23. The disulfide bridge built into the spacer arm of DTBP allows for easy cleavage of a protein conjugate using standard reducing agents.

Spacer arm structure

Crosslinkers typically possess a straight chain spacer arm, but protein modification reagents allow more options. A good example is our PEGylation reagents which can be either straight or branched. For example, CA(PEG) is a straight chain PEGylation reagents and TMM(PEG)$_{12}$ is a branched reagent (Figure 24).

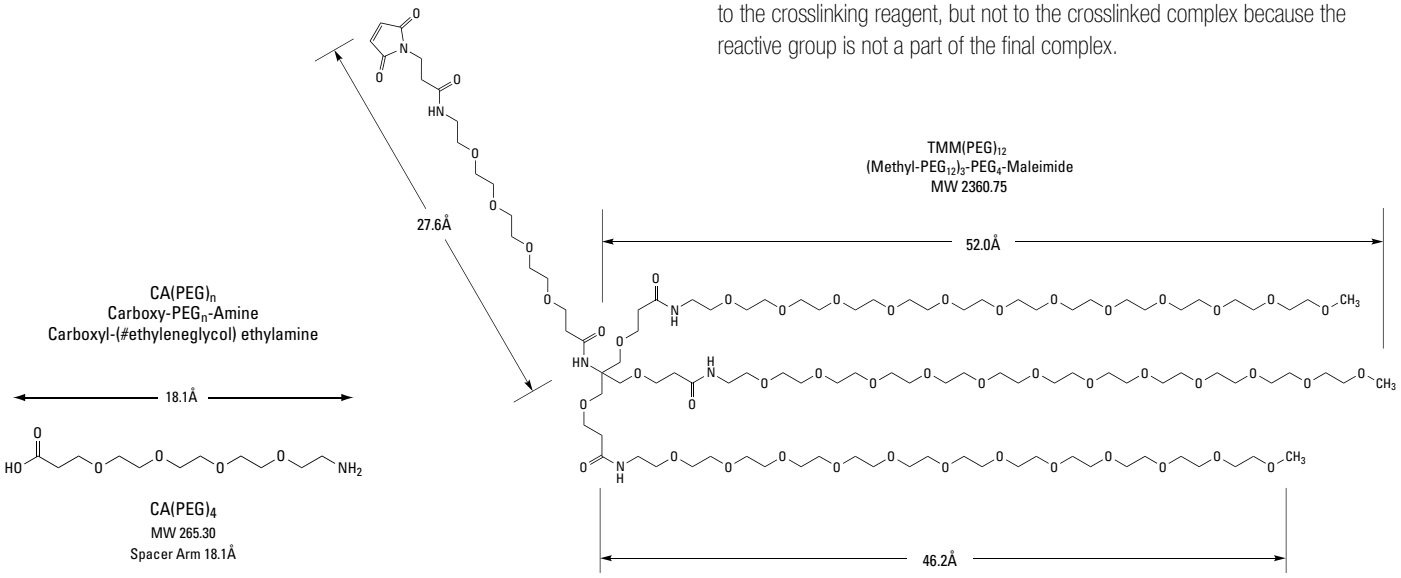


Figure 24. Straight and branched protein modification reagents.

Water solubility and cell membrane permeability

Many crosslinkers, by virtue of their hydrophobic spacer arms, have limited solubility in aqueous solutions. These crosslinkers are generally dissolved in DMF or DMSO, then added to the biological system or solution of biomolecules to be crosslinked. Hydrophobic crosslinkers are able to cross cellular and organellar membranes and affect crosslinking both at the outer surface of a membrane and within the membrane-bounded space.

It is often inconvenient or undesirable to introduce organic solvents into a crosslinking procedure for a biological system. It is also desirable in many instances to affect crosslinking only on the outer surface of a cellular or organellar membrane without altering the interior of the cell or organelles. For such cases, several water-soluble, membrane-impermeable crosslinkers are available.

Some crosslinkers contain a spacer arm formed from PEG subunits, resulting in a polyethylene oxide (PEO) chain with abundant oxygen atoms to provide water solubility. These crosslinkers are designated by a $(PEG)^n$ in their name and are both water-soluble and unable to penetrate biological membranes. They provide the added benefit of transferring their hydrophilic spacer to the crosslinked complex, decreasing the potential for aggregation and precipitation of the complex.

Other crosslinkers obtain their water-solubility and membrane-impermeability by virtue of a charged reactive group at either end of the spacer. These charged reactive groups, such as sulfo-NHS esters or imidoesters, impart water-solubility to the crosslinking reagent, but not to the crosslinked complex because the reactive group is not a part of the final complex.

conjugate
bridging chemistry and biology

Crosslinkers and protein modification reagents have a variety of applications in life science research and assay development. Below are some examples of how researchers have used bioconjugation chemistry for various purposes.

Creating protein conjugates

Protein conjugates are often designed to enable purification and detection in a complex biological sample. Commonly, antibodies are the target of bioconjugation with common labels such as biotin or chemically reactive fluorescent dyes such as Thermo Scientific DyLight Dyes (Figure 25). In this process, NHS ester chemistry is the most widely used method for labeling available lysine residues. For more information on biotinylation, refer to the free Avidin-Biotin Technical Handbook (# 1601675).

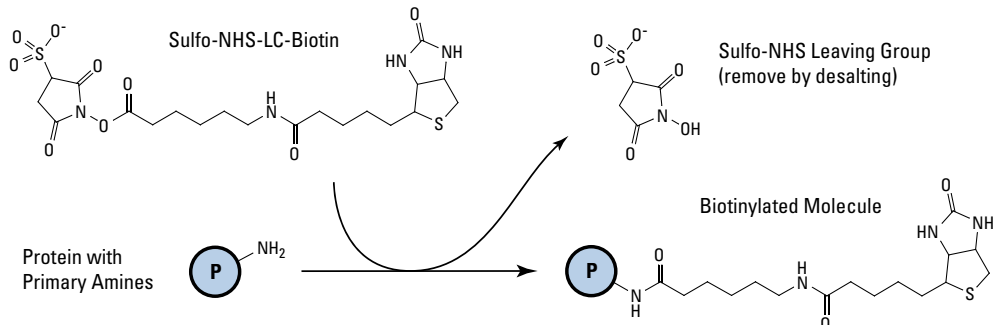


Figure 25. Biotinylation using NHS ester chemistry.

Similar chemistry is used to label biomolecules with DyLight™ Fluorescent Dyes for detection by fluorescent microscopy, flow cytometry, high content analysis, high density arrays, *in vivo* imaging, immunoblotting and immunostaining. Fluorescent dyes are available activated with NHS ester chemistry or malemide chemistry for labeling at primary amines and reduced sulfhydryls, repectively (Figure 26).

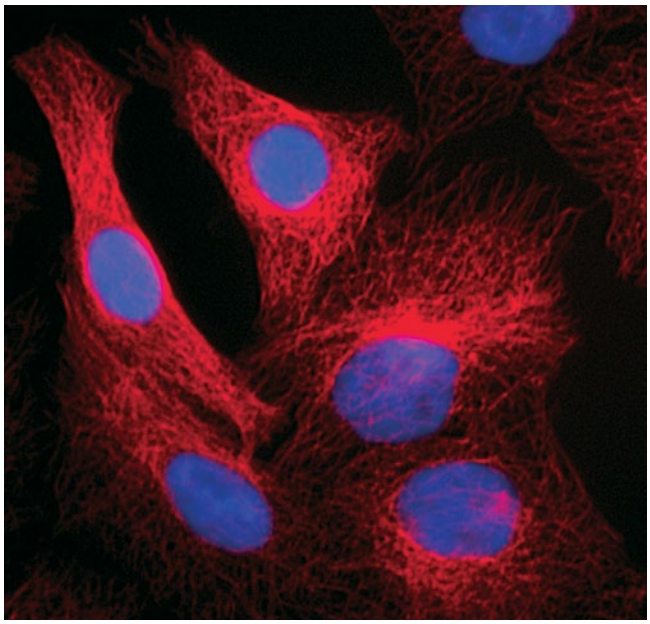


Figure 26. The Thermo Scientific NHS-Rhodamine Antibody Labeling Kit (Product # 53031) produces ideal conjugates for immunofluorescence. A549 cells were fixed with 4% paraformaldehyde (Product # 28906) and permeabilized with 0.1% Surfact-Amps™ X-100 (Product # 28314). The cells were then probed with a 0.4µg/mL mouse anti-α-tubulin antibody and 2µg/mL rhodamine-goat anti-mouse secondary antibody. Nuclei were labeled with Hoechst 33342. Images were acquired on Nikon Eclipse™ TS100 fluorescent microscope using Zeiss AxioCam™ camera and AxioVision™ software.

Many crosslinkers are used for making conjugates for use as immunogens. The best crosslinker to use depends on the functional groups present on the hapten and the ability of the hapten-carrier conjugate to function successfully as an immunogen after its injection. Carbodiimides are good choices for producing peptide-carrier protein conjugates because both proteins and peptides usually contain several carboxyls and primary amines. Carbodiimides such as EDC react with carboxyls first to yield highly reactive unstable intermediates that can then couple to primary amines. Often peptides are synthesized with terminal cysteines to enable attachment to supports or to carrier proteins using sulfhydryl-/amine-reactive, heterobifunctional crosslinkers (Figure 27). This method can be very efficient and yield an immunogen that is capable of eliciting a good response upon injection. For more information on preparation of immunogen conjugates, refer to the free Antibody Production and Purification Technical Handbook (1601974).

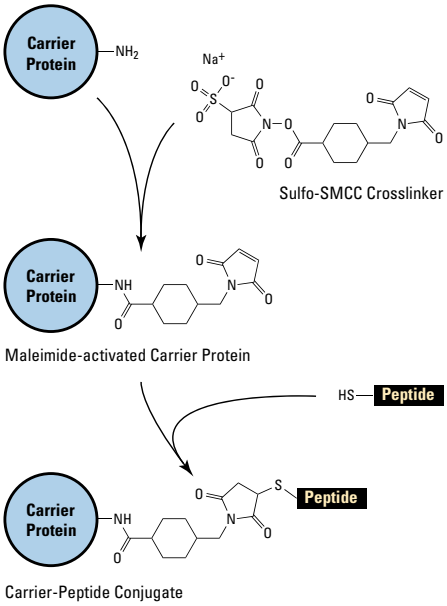


Figure 27. Peptide conjugation to carrier proteins for antibody production.

One of the most common applications for crosslinkers is the production of protein:protein conjugates. Conjugates are often prepared by attachment of an enzyme, fluorophore or other molecule to a protein that has affinity for one of the components in the biological system being studied. Antibody-enzyme conjugates (primary or secondary antibodies) are among the most common protein:protein conjugates used. Although secondary antibody conjugates are available and relatively inexpensive, enzyme-labeled primary antibodies are usually expensive and can be difficult to obtain. Many reagents are used for the production of antibody-enzyme conjugates. Glutaraldehyde conjugates are easy to make, but they often yield conjugates that produce high background in immunoassays. Carbohydrate moieties can be oxidized and then coupled to primary amines on enzymes in a procedure called reductive alkylation or amination. These conjugates often result in less background in enzyme immunoassays and are relatively easy to prepare; however, some self-conjugation of the antibody may occur (Figure 28).

Homobifunctional NHS ester or imidoester crosslinkers may be used in a one-step protocol but polymerization and selfconjugation are also likely. Homobifunctional sulfhydryl-reactive crosslinkers such as BMH (Product # 22330) may be useful if both proteins to be conjugated contain sulfhydryls. Heterobifunctional crosslinkers are perhaps the best choices for antibody-enzyme or other protein:protein crosslinking. Unwanted self-conjugation inherent when using homobifunctional NHS ester reagents or glutaraldehyde can be avoided by using a reagent such as SMCC (Product # 22360) or Sulfo-SMCC (Product # 22322). Sulfo-SMCC is first conjugated to one protein, and the second is thiolated with SATA (Product # 26102) or Traut's Reagent (Product # 26101), followed by conjugation (Figure 29). Alternatively, disulfides in the protein may be reduced, and the two activated proteins are incubated together to form conjugates free of dimers of either protein. Any of the other NHS ester, maleimide or pyridyl disulfide crosslinkers can be substituted for Sulfo-SMCC in this reaction scheme. Heterobifunctional photoactivatable phenyl azide crosslinkers are seldom used for making protein:protein conjugates because of low conjugation efficiencies.

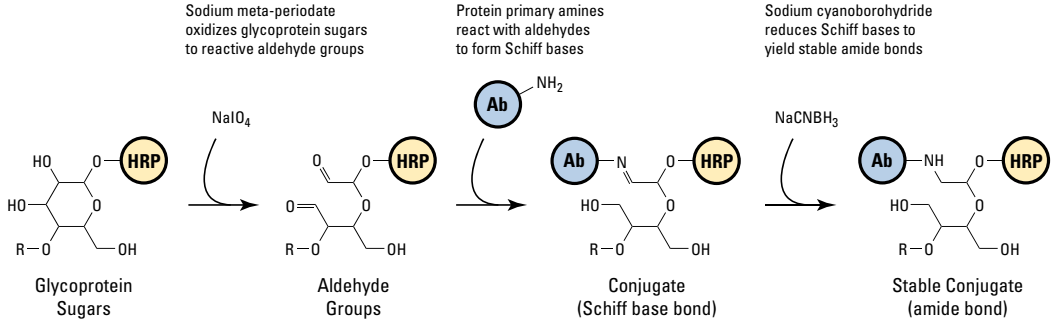


Figure 28. Reaction scheme for labeling antibodies with enzymes such as HRP using reductive animation.

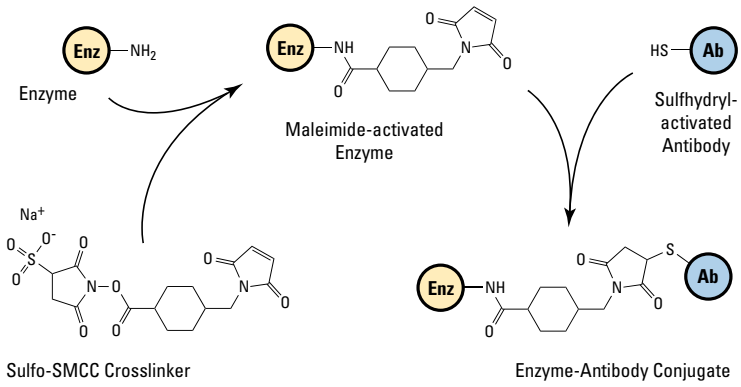


Figure 29. Reaction scheme for labeling reduced antibody fragments with maleimide-activated enzymes.

Protein immobilization onto solid supports

Proteins, peptides and other molecules can be immobilized onto solid supports for affinity purification of proteins or for sample analysis. The supports may be nitrocellulose or other membrane materials, polystyrene plates or beads, agarose, beaded polymers, or glass slides. Some supports can be activated for direct coupling to a ligand. Other supports are made with nucleophiles or other functional groups that can be linked to proteins using crosslinkers. Carbodiimides such as DCC (Product # 20320) and EDC (Product # 22980, 22981) are very useful for coupling proteins to carboxy- and amine-activated glass, plastic and agarose supports. Carbodiimide procedures are usually one-step methods; however, two-step methods are possible if reactions are performed in organic solvents, or if NHS (Product # 24500) or Sulfo-NHS (Product # 24510) is used to enhance the reaction. EDC is useful for coupling ligands to solid supports and to attach leashes onto affinity supports for subsequent coupling of ligands. Useful spacers are diaminodipropylamine (DADPA), ethylenediamine, hexanediamine, 6-amino-caproic acid and any of several amino acids or peptides. Spacer arms help to overcome steric effects when the ligand is immobilized too near the matrix to allow access by the receptor. Steric effects are usually most pronounced when the ligand is a small molecule. Our aldehyde-activated AminoLink™ Plus Agarose Resin (Product # 20501) uses reductive amination, NHS-activated agarose (Product # 26200) uses NHS ester chemistry, and our SulfoLink™ Resin (Product # 20401) uses haloacetyl chemistry to immobilize molecules, whereas our CarboxyLink Resin (Product # 20266) uses carbodiimide chemistry. (Figure 30).

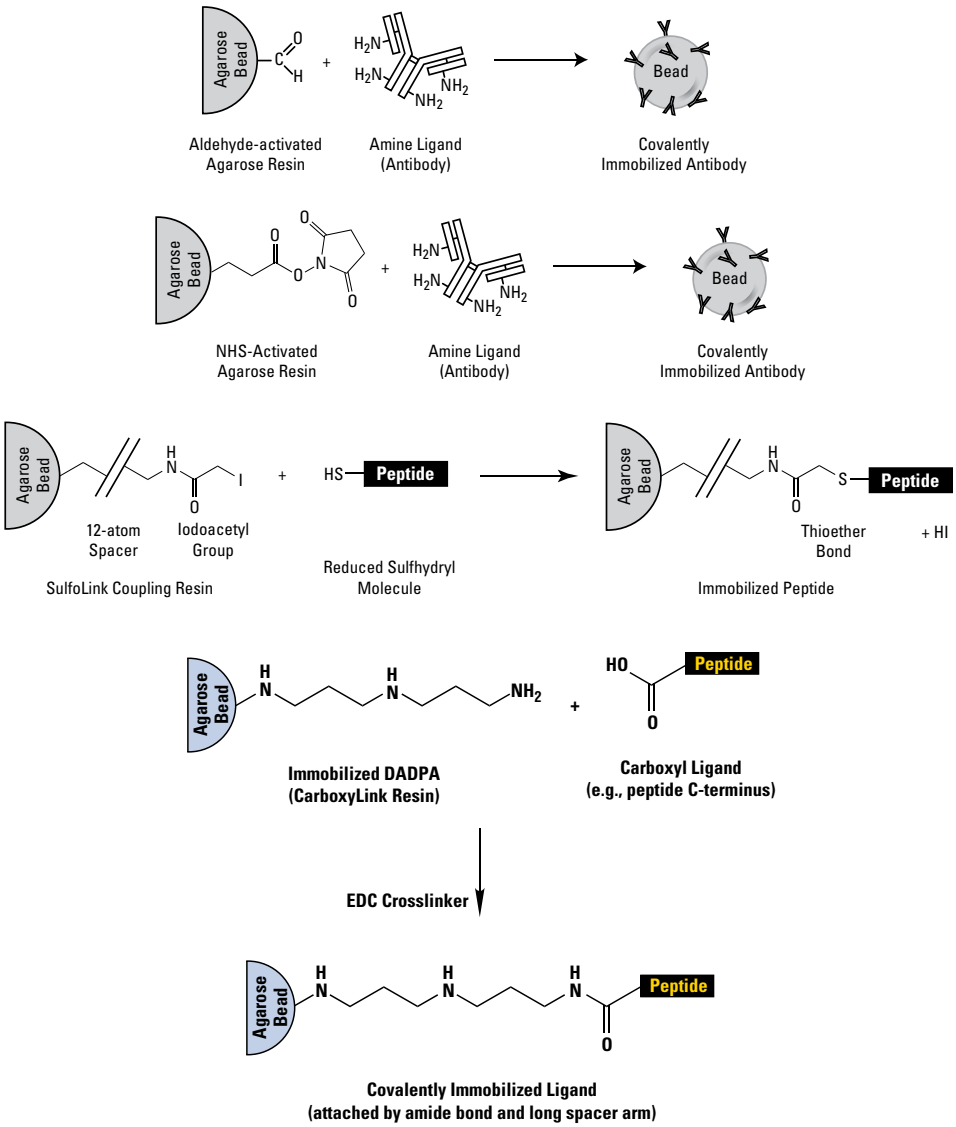
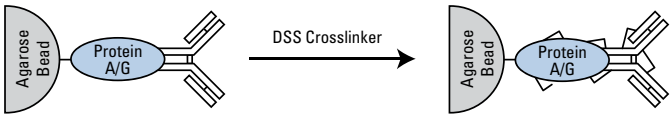


Figure 30. Immobilization of biomolecules onto solid supports using different bioconjugation chemistry.

The crosslinkers DMP (Product # 21666) and DSS (Product # 21555) are used to immobilize antibodies on Protein A or Protein G supports for antigen purification. After the antibody binds to the Fc-binding proteins, the antibody is oriented so that the Fab region is available for antigen binding. DSS or DMP is applied to the bound antibody column to link the two proteins through primary amines. Thermo Scientific Crosslink IP Kit (Product # 26147) is based on this chemistry. For more information on solid-phase immobilization, refer to the free Protein Purification Technical Handbook (Product # 1602015).



In vivo crosslinking

Crosslinkers are used for identification of near-neighbor protein relationships and ligand-receptor interactions. Crosslinking stabilizes transient endogenous protein:protein complexes which may not survive traditional biochemical techniques such as immunoprecpitation. The most basic cellular crosslinker is formaldehyde (Product # 28906) which is commonly used to stabilize chromatin interactions for Chromatin Immunoprecipitation (ChIP) assays (Product # 26156). The crosslinkers chosen for these applications are usually longer than those used for subunit crosslinking. Homobifunctional, amine-reactive NHS esters or imidates and heterobifunctional, amine-reactive, photoactivatable phenyl azides are the most commonly used crosslinkers for these applications. Occasionally, a sulfhydryland amine-reactive crosslinker, such as Thermo Scientific Sulfo-SMCC (Product # 22322), may be used if one of the two proteins or molecules is known to contain sulfhydryls. Both cleavable or noncleavable crosslinkers can be used. Because the distances between two molecules are not always known, the optimal length of the spacer arm of the crosslinker may be determined by the use of a panel of similar crosslinkers with different lengths. Thermo Scientific DSS (Product # 21555) or its cleavable analog DSP (Product # 22585) are among the shorter crosslinkers used for protein:protein interactions.

In contrast to crosslinkers which are introduced to cells exogenously, methods exist to incorporate crosslinkers into the proteome of a cell. This can be accomplished with photo-reactive crosslinkers such as L-Photo-Leucine (Product # 22610) and L-Photo-Methionine (Product # 22615). These amino acid analogues are fed to cells during cell growth and are activated with UV light. In the experiment below, photo-reactive amino acids were compared to formaldehyde treatment for identifying endogenous protein complexes (Figure 32).

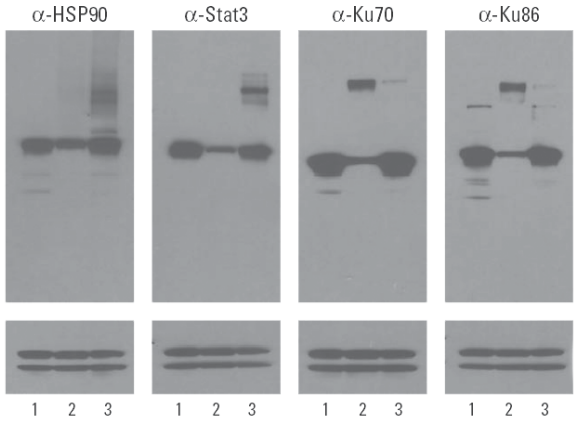


Figure 32. Photo-reactive amino acid crosslinking and formaldehyde crosslinking are complementary techniques for protein interaction analysis. HeLa cells were mocktreated (Lane 1), treated with 1% formaldehyde for 10 minutes (Lane 2), or treated with Photo-Methionine and Photo-Leucine followed by UV treatment (Lane 3). Cells were lysed and 10µg of each was analyzed by SDS-PAGE and Western blotting with antibodies against HSP90, STAT3, Ku70 and Ku86. Lower panels are beta-actin (upper band) and GAPDH (lower band), which were blotted as loading controls.

Reference:

Suchanek, M., *et al.* (2005). Photo-leucine and photo-methionine allow identification of protein-protein interactions. *Nat. Methods* **2(4),** 261-267.

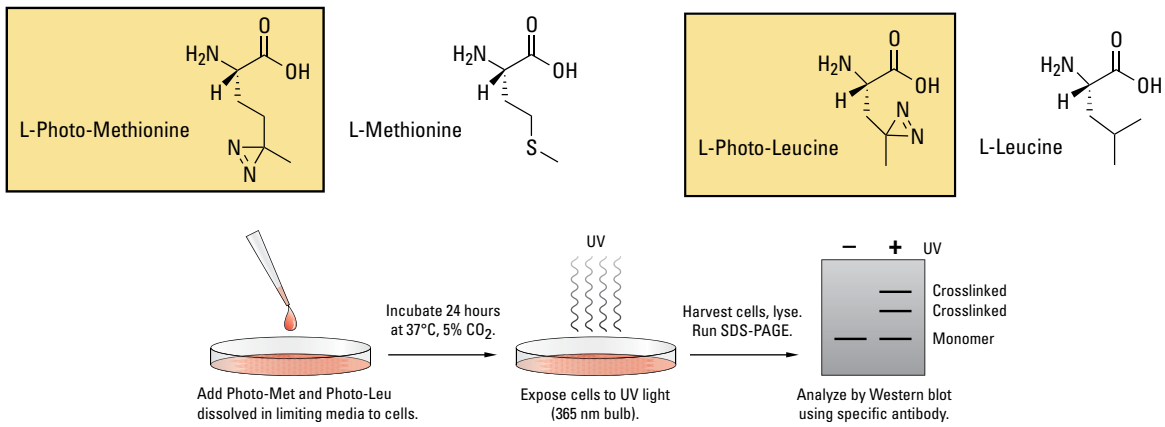


Figure 31. *In vivo* crosslinking with photo-reactive amino acids.

Creation of immunotoxins

Specific antibodies can be covalently linked to toxic molecules and then used to target antigens on cells. Often these antibodies are specific for tumor-associated antigens. Immunotoxins are brought into the cell by surface antigens and, once internalized, they proceed to kill the cell by ribosome inactivation or other means. The type of crosslinker used to make an immunotoxin can affect its ability to locate and kill the appropriate cells. For immunotoxins to be effective, the conjugate must be stable *in vivo*. In addition, once the immunotoxin reaches its target, the antibody must be separable from the toxin to allow the toxin to kill the cell. Thiol-cleavable, disulfide-containing conjugates have been shown to be more cytotoxic to tumor cells than noncleavable conjugates of ricin A immunotoxins. Cells are able to break the disulfide bond in the crosslinker, releasing the toxin within the targeted cell.

Thermo Scientific SPDP (Product # 21857) is a reversible NHS ester, pyridyl disulfide crosslinker used to conjugate amine containing molecules to sulfhydryls. For several years, this has been the "workhorse" crosslinker for production of immunotoxins. The amine-reactive NHS ester is usually reacted with the antibody first. In general, toxins do not contain surface sulfhydryls; therefore, sulfhydryls must be introduced into them by reduction of disulfides, which is common for procedures involving ricin A chain and abrin A chain, or through chemical modification reagents. A second SPDP molecule can be used for this purpose and is reacted with amines on the immunotoxin, then reduced to yield sulfhydryls.

Another chemical modification reagent that is commonly used for production of immunotoxins is 2-iminothiolane, also known as Traut's Reagent (Product # 26101). Traut's Reagent reacts with amines and yields a sulfhydryl when its ring structure opens during the reaction.

Label transfer

Label transfer involves crosslinking interacting molecules (i.e., bait and prey proteins) with a labeled crosslinking agent and then cleaving the linkage between bait and prey such that the label remains attached to the prey (Figure 34, page 21). This method allows a label to be transferred from a known protein to an unknown, interacting protein. The label can then be used to purify and/or detect the interacting protein. Label transfer is particularly valuable because of its ability to identify proteins that interact weakly or transiently with the protein of interest. New non-isotopic reagents and methods continue to make this technique more accessible and simple to perform by any researcher.

• Sulfo-SBED

Label transfer reagents can also have biotin built into their structure. This type of design allows the transfer of a biotin tag to an interacting protein after cleavage of a cross-bridge. Sulfo-SBED (Product # 33033) is an example of such a trifunctional reagent (Figure 33). It contains an amine-reactive sulfo-NHS ester on one arm (built off the α-carboxylate of the lysine core), a photo-reactive phenyl azide group on the other side (synthesized from the α-amine) and a biotin handle (connected to the ε-amino group of lysine). The arm containing the sulfo-NHS ester has a cleavable disulfide bond, which permits transfer of the biotin component to any captured proteins.

In use, a bait protein first is derivatized with Sulfo-SBED through its amine groups, and the modified protein is allowed to interact with a sample. Exposure to UV light (300-366nm) couples the photo-reactive end to the nearest available C-H or N-H bond in the bait:prey complex, resulting in covalent crosslinks between bait and prey. Upon reduction and cleavage of the disulfide spacer arm, the biotin handle remains attached to the protein(s) that interacted with the bait protein, facilitating isolation or identification of the unknown species using streptavidin, Thermo Scientific NeutrAvidin Protein or monomeric avidin.

The architecture of this trifunctional label transfer reagent differs substantially from the bifunctional counterparts discussed above. The advantages become almost immediately apparent just by examining the structure.

The reactive moieties are well-segregated within Sulfo-SBED. Most importantly, with a biotin label designed into Sulfo-SBED, radiolabeling with ^{125}I is no longer necessary. The biotin label can be used to significant advantage in a label transfer application. For example, biotin can operate as a handle for purification of the prey protein or prey protein fragments or as a detection target using streptavidin-HRP and colorimetric or chemiluminescent substrates.

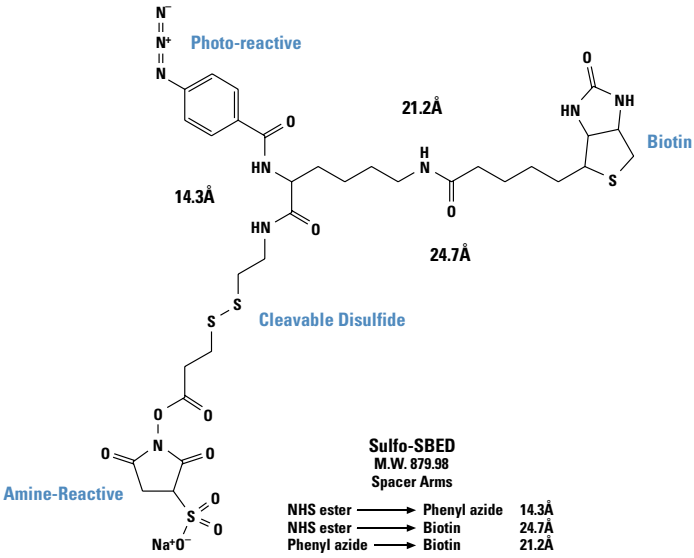


Figure 33. Structure of Sulfo-SBED.

• Applications for Sulfo-SBED

Since the first availability of this reagent in 1994, the number of literature references for use of Sulfo-SBED in protein interaction-related applications has grown rapidly. Published applications show how Sulfo-SBED can used to:

- Define interactions of complexes with activator domains[1]
- Clarify the mechanism of protein complex assembly[2]
- Convert to a sulfhydryl-reactive trifunctional reagent to map interactions[3]
- Study docking site and factor requirements for binding[4]
- Describe binding contacts of interactors[5]
- Confirm recognition of a specific phosphoepitope[6]
- Search for putative binding partners[7]
- Gain insight into chaperone-mediated refolding interactions[8]
- Investigate mechanism of protein interaction[9]
- Facilitate receptor activity-directed affinity tagging (re-tagging)[10]
- Detect low-abundance protein receptors
- Find protein:carbohydrate interactions
- Understand drug-receptor interactions[11]
- Quantitate triple helix-forming oligonucleotides[12]

Routes to determining the prey protein identification using Sulfo-SBED are outlined schematically in Figure 34. Note that the biotin label is a purification handle for captured prey protein. In the trypsin digestion strategy, the peptide(s) trapped can offer information relating to the binding interaction interface. The biotin-labeled prey protein or prey protein peptides recovered as result of the strategies outlined below can be subjected to several detection and identification options designed to discover the identity of the prey protein.

References

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11. Jacobson, K.A., *et al.* (1995). *Life Sciences* **56** (11/12), 823-830.
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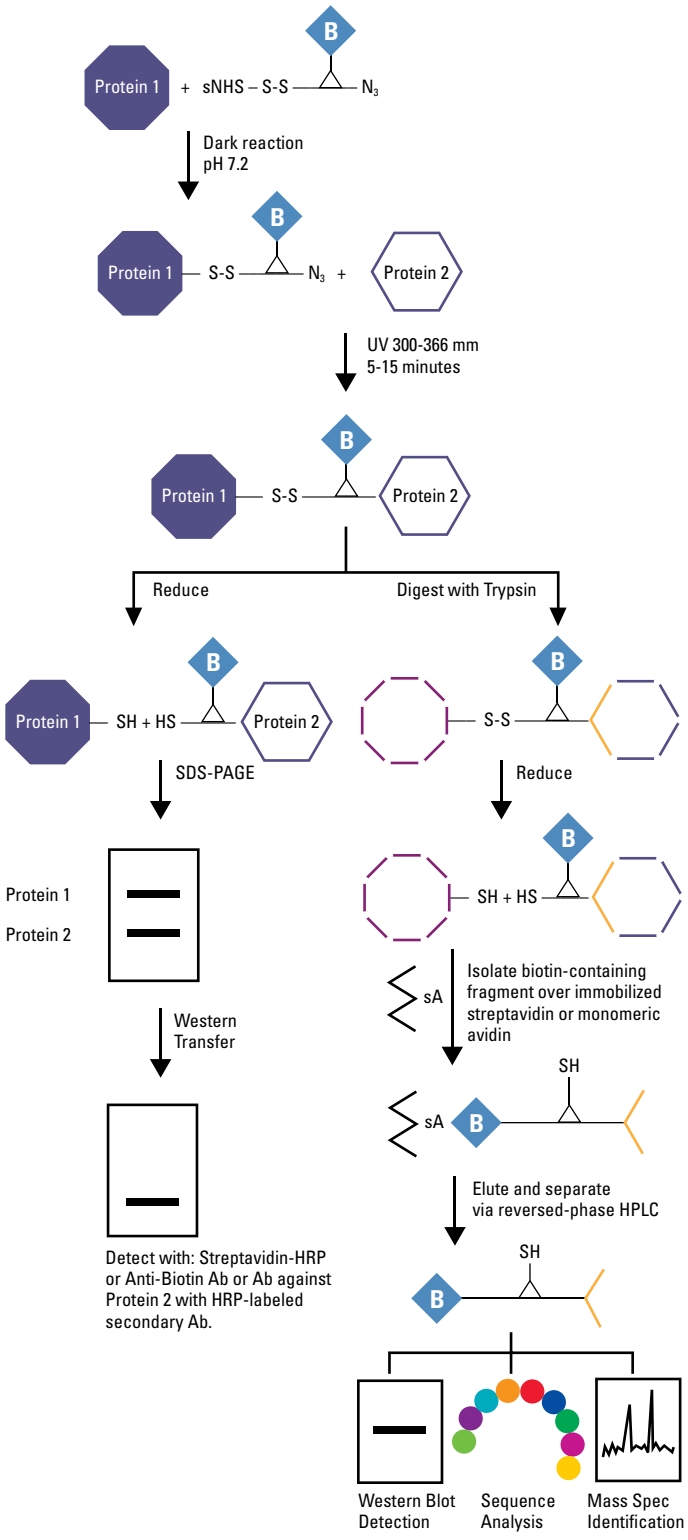


Legend:

sNHS	S-S	N_3	B
Sulfo *N*-Hydroxy succinmide ester	Disulfide bond	Phenyl azide	Biotin

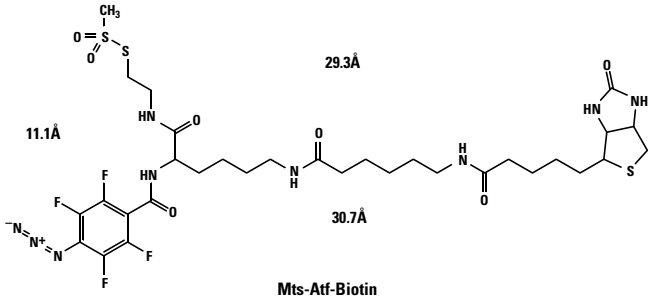
Figure 34. Applications of Sulfo-SBED in protein interaction studies.

• MTS-Atf-Biotin and MTS-Atf-LC-Biotin

These two Pierce Biotin-containing Reagents incorporate the benefits of the sulfhydryl-specific methanethiosulfonate (Mts) group and the high-yielding photoreactive tetrafluorophenyl-azide moiety. By combining these reactive groups with a biotin tag, powerful new reagents for protein interaction analysis were created. Purified bait protein is labeled at reduced cysteine residues, then allowed to form an interaction complex with the prey protein. When exposed to UV-light, the photoreactive group activates to form covalent bonds to adjacent sites on the prey protein. Reducing the disulfide-bond releases the bait protein and leaves the biotin label on the prey.

Highlights:

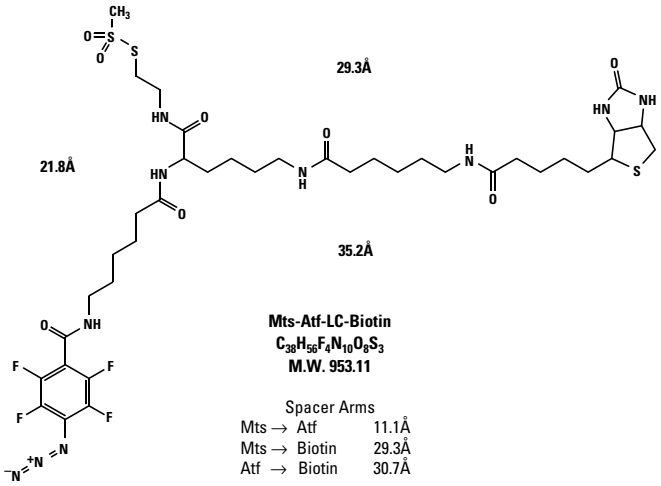
- Mts moiety is highly specific for the sulfhydryl (–SH) group that occurs in the side chain of reduced cysteine residues, enabling precise, rapid and quantitative labeling of the bait protein
- Tetrafluorophenyl azide moiety reacts three- to four-times more efficiently than regular phenyl azide moieties, increasing the likelihood of capturing sufficient bait:prey complex to detect
- Sulfinic acid byproducts of the Mts reaction do not interfere with disulfide bond formation or the activity of the bait protein and decomposes quickly to a volatile low molecular weight product
- Disulfide bond spacer arm connecting bait and prey proteins is easily reversed with commonly used reducing agents DTT, 2-mercaptoethanol or TCEP
- Mts reaction and photoreaction are compatible with physiologic buffer conditions required for most protein interactions
- Long chain (LC) and short chain versions are offered, allowing one to more precisely explore interaction distances



Mts-Atf-Biotin
$C_{32}H_{45}F_4N_9O_7S_3$
M.W. 839.95

Spacer Arms

Mts →	Atf	11.1Å
Mts →	Biotin	29.3Å
Atf →	Biotin	30.7Å



Mts-Atf-LC-Biotin
$C_{38}H_{56}F_4N_{10}O_8S_3$
M.W. 953.11

Spacer Arms

Mts →	Atf	11.1Å
Mts →	Biotin	29.3Å
Atf →	Biotin	30.7Å

Structure determination with heavy/light crosslinker pairs

Recently, chemical crosslinking, combined with high-resolution mass spectrometry, has emerged as a strategy to obtain low-resolution three-dimensional structural data of protein structures and protein interfaces in complexes from low quantities of proteins within a relatively short time. However, identification of the large number of crosslinking sites from the complex mixtures generated by chemical crosslinking remains a challenging task.

By incorporating an isotopic label into the crosslinking reagent, thus conducting linking and labeling in one step, the crosslinked peptides are identified easily in the presence of the numerous unmodified tryptic peptides. The strategy requires the availability of both "light" or hydrogen-containing and "heavy" or discretely substituted deuterium analogs of crosslinking agents. Heavy and light analogs are reacted simultaneously with the target protein or protein complex. Use of heavy and light crosslinkers in this application dramatically simplifies identification of the peptides resulting from the coupling reactions. Application of a 1:1 ratio of two identical crosslinking agents differing only in the number of deuterium atoms in their chemical composition (e.g., d_4 vs d_0) facilitates identification of low-abundance crosslinked peptides. Isotopic MS patterns, differing by four mass units after enzymatic digestion of the crosslinked protein or protein complex, identifies the crosslinked products.

Further analysis of the reaction products resulting from the simultaneous reaction of these heavy and light crosslinkers with a target protein or protein complex is accomplished by MALDI-TOF-MS, ESI-LC/MS/MS or ESI-FTICR-MS. The results positively identify the crosslinked peptides. Distance constraints provided by these data can yield low-resolution three-dimensional structure information that can be used to create structural models of the protein in solution. Intermolecular crosslinking of an interacting protein complex and subsequent MS analysis have been successfully applied to determine the contact surfaces of binding partners in a protein complex.[1-5]

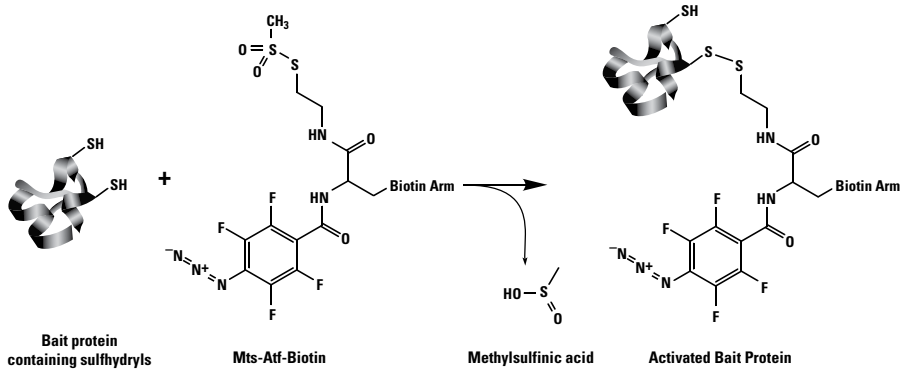
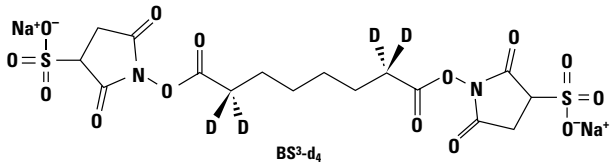


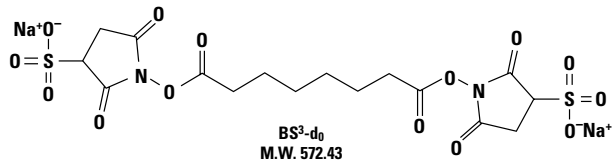
Figure 35. Reaction of Mts-Atf-Biotin with bait protein containing sulfhydryls (reduced disulfide bonds). Once desalted to remove excess nonreacted Mts-Atf-Biotin and byproducts (methylsulfinic acid), the activated bait protein may be allowed to interact with other proteins (the prey) and then crosslinked together by UV-activation of the tetrafluorophenyl azide group. If desired, the disulfide bond in the Mts-Atf-Biotin may be cleaved with a reducing agent, transferring the biotin label to the prey protein.

• Heavy/light crosslinker pairs

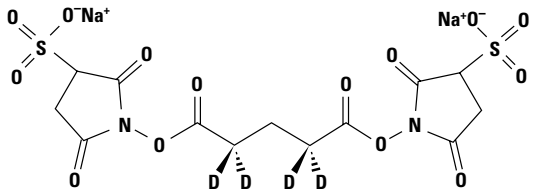
Thermo Scientific Pierce BS^2G and BS^3 are water-soluble, homobifunctional sulfonated N-hydroxysuccinimide esters (Sulfo-NHS esters) with a 7.7Å and 11.4Å spacer arm that can act as molecular rulers for estimation of spatial relationships in protein structure-function studies. The reagents described here are the deuterated and non-deuterated analogs of BS^2G and BS^3. These reagents react efficiently with primary amine groups ($–NH_2$) at pH 7-9 to form stable amide bonds. Proteins generally contain several primary amines in the form of lysine residue side chains and the N-terminus of each polypeptide that are available as targets for the NHS ester-reactive group. BS^2G-d_4 and BS^3-d_4 react identically to their H-substituted counterparts. These reagents are supplied as a sodium salt and are soluble in water at a concentration up to 10mM.



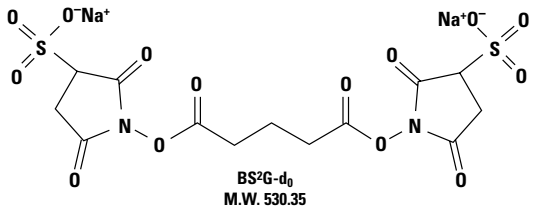
BS^3-d_4
M.W. 576.45
Spacer Arm 11.4Å



BS^3-d_0
M.W. 572.43
Spacer Arm 11.4Å



BS^2G-d_4
M.W. 534.38
Spacer Arm 7.7Å



BS^2G-d_0
M.W. 530.35
Spacer Arm 7.7Å

Figure 36. Heavy BS^3 antibodies.

References:

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Metabolic labeling

Chemoselective ligations use unique chemical functional groups for specific conjugation. Examples of this chemistry include hydrazide-aldehyde condensation, click chemistry (azide-alkyne) and Staudinger ligation (azide-phosphine).[1] Of these reactions,the Staudinger ligation has the best utility for live-cell labeling and mass spectrometry (MS) applications. The Staudinger reaction occurs between a phosphine and an azide to produce an aza-ylide that is trapped to form a stable covalent bond. Because phosphines and azides are absent in biological systems, there is minimal background labeling of cells or lysates. Unlike click chemistry, Staudinger ligation requires no accessory reagents such as copper.

Metabolic labeling involves incorporation of a chemoselective crosslinker into the proteome of living cells. This facilitates protein isolation or fluorescent labeling. One such example is the metabolic labeling of glycoproteins using azido sugars. Azido groups react with phosphines to create a stable amide bond following the Staudinger reaction. Once incorporated, azido sugars can be labeled with biotin (Figure 37) or DyLight fluorophores (Figures 38-39). In the examples below, U2OS cells or HK-2 cells were fed azido sugars, fixed and stained with Dylight 550-Phosphine (Product # 88910) or DyLight 650-Phosphine (Product # 88911).

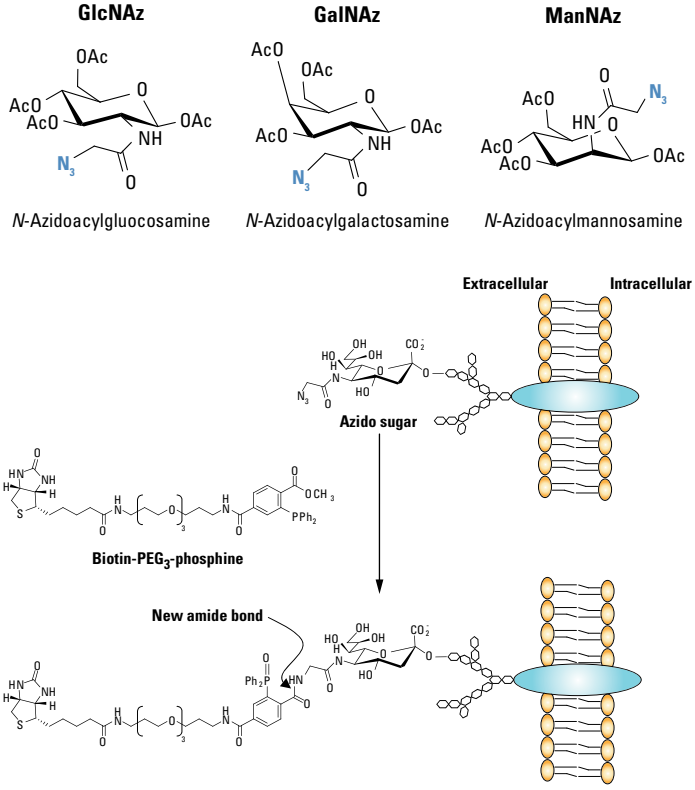


Figure 37. Biotin labeling of azido sugars.

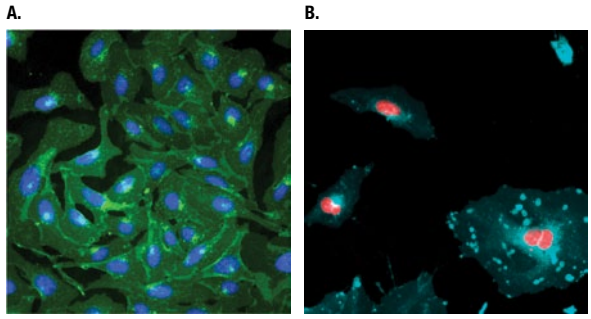


Figure 38. *In vivo* detection of metabolically incorporated azido-acetylgalactosamine using Thermo Scientific DyLight 550- and 650-Phosphine Labeling Reagents. Panel A. U2OS cells were incubated with 40µM azido-acetylgalactosamine in cell culture media for 72 hours and the live cells were incubated with 100µM of DyLight 550-phosphine . The cells were then washed, fixed with 4% paraformaldehyde and counterstained with Hoechst 33342. (green: DyLight 550-labeled azido-galactosamine, blue: Hoechst 33342 labeled nuclei). **Panel B.** HK-2 cells were incubated with 40µM azido-acetylmannosamine in cell culture media for 72 hours and the live cells were incubated with 100µM of DyLight 650-phosphine. The cells were then washed, fixed with 4% paraformaldehyde and counterstained with Hoechst 33342. (cyan: DyLight 650-labeled azido-mannosamine, red: Hoechst 33342-labeled nuclei).

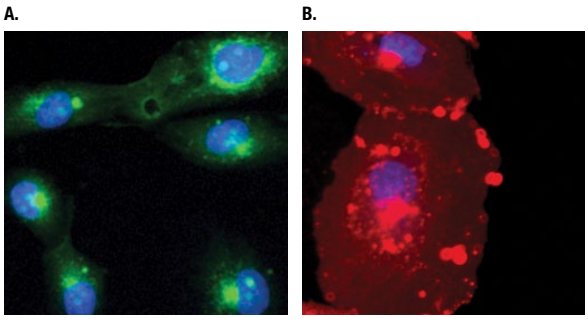


Figure 39. Detection of metabolically incorporated azido-sugars on live and fixed cells using Thermo Scientific DyLight DyLight 550- and 650-Phosphine Labeling Reagents. HK-2 cells were incubated with 40µM azido-acetylmannosamine in cell culture media for 72 hours. The azido-sugar were labeled either after 4% paraformaldehyde fixation using DyLight 550-phosphine (Panel A) or labeled in live cells with DyLight 650-Phosphine Labeling Reagent (Panel B). The cells were washed and counterstained with Hoechst 33342. **Panel A.** The golgi structure (green) is predominantly detected by fixed cell labeling, where as the cell membrane and secretory vesicles (red) are labeled by live-cell labeling (blue: Hoechst 33342-labeled nuclei).

Cell surface crosslinking

Crosslinkers are often used to identify surface receptors or their ligands. Membrane-impermeable crosslinkers ensure cell-surface-specific crosslinking. Water-insoluble crosslinkers when used at controlled amounts of reagent and reaction time can reduce membrane penetration and reaction with inner membrane proteins.

The sulfonyl groups attached to the succinimidyl rings of NHS esters result in a crosslinker that is water-soluble, membrane-impermeable and nonreactive with inner-membrane proteins. Therefore, reaction time and quantity of crosslinker are less critical when using sulfo-NHS esters. Homobifunctional sulfo-NHS esters, heterobifunctional sulfo-NHS esters and photoreactive phenyl azides are good choices for crosslinking proteins on the cell surface.

Determination of whether a particular protein is located on the surface or the integral part of the membrane can be achieved by performing a conjugation reaction of a cell membrane preparation to a known protein or radioactive label using a water-soluble or water-insoluble crosslinker. Upon conjugation the cells may be washed, solubilized and characterized by SDS-polyacrylamide gel electrophoresis (PAGE) to determine whether the protein of interest was conjugated. Integral membrane proteins will form a conjugate in the presence of a water-insoluble crosslinker, but not in the presence of water-soluble crosslinkers. Surface membrane proteins can conjugate in the presence of water-soluble and water-insoluble crosslinkers. Thermo Scientific BASED (Product # 21564), a homobifunctional photoactivatable phenyl azide, is one of the more versatile crosslinkers for the study of protein interactions and associations. It is cleavable and can be radiolabeled with ^{125}I using Thermo Scientific Pierce Iodination Beads (Product # 28665). After cleavage, both of the dissociated molecules will still be iodinated. Because both reactive groups on this crosslinker are nonspecific, the crosslinking is not dependent on amino acid composition for successful conjugation.

Cell membrane structural studies

Cell membrane structural studies require reagents of varying hydrophobicity to determine the location and the environment within a cell's lipid bilayer. Fluorescent tags are used to locate proteins, lipids or other molecules inside and outside the membrane. Various crosslinkers, with differing spacer arm lengths, can be used to crosslink proteins to associated molecules within the membrane to determine the distance between molecules. Successful crosslinking with shorter crosslinkers is a strong indication that two molecules are interacting in some manner. Failure to obtain crosslinking with a panel of shorter crosslinkers, while obtaining conjugation with the use of longer reagents, generally indicates that the molecules are located in the same part of the membrane, but are not interacting. Homobifunctional NHS esters, imidates or heterobifunctional NHS ester/photoactivatable phenyl azides are commonly used for these procedures. Although imidoester crosslinkers (imidates) are water-soluble, they are still able to penetrate membranes. Sulfhydryl-reactive crosslinkers may be useful for targeting molecules with cysteines to other molecules within the membrane.

Thermo Scientific EDC (Product # 22980, 22981), water-insoluble dicyclohexylcarbodiimide (DCC, Product # 20320) and other water-soluble/-insoluble coupling reagent pairs are used to study membranes and cellular structure, protein subunit structure and arrangement, enzyme:substrate interactions, and cell-surface and membrane receptors. The hydrophilic character of EDC can result in much different crosslinking patterns in membrane and subunit studies than with hydrophobic carbodiimides such as DCC. Often it is best to attempt crosslinking with a water-soluble and water-insoluble carbodiimide to obtain a complete picture of the spatial arrangements or protein:protein interactions involved.

Subunit crosslinking and protein structural studies

Crosslinkers can be used to study the structure and composition of proteins in samples. Some proteins are difficult to study because they exist in different conformations with varying pH or salt conditions. One way to avoid conformational changes is to crosslink subunits. Amine-, carboxyl- or sulfhydryl-reactive reagents are used for identification of particular amino acids or for determination of the number, location and size of subunits. Short- to medium-spacer arm crosslinkers are selected when intramolecular crosslinking is desired. If the spacer arm is too long, intermolecular crosslinking can occur. Carbodiimides that result in no spacer arm, along with short-length conjugating reagents, such as amine-reactive Thermo Scientific DFDNB (Product # 21525) or the photoactivatable amine-reactive crosslinker Thermo Scientific NHS-ASA (Product # 27714), can crosslink between subunits without crosslinking to extraneous molecules if used in optimal concentrations and conditions. Slightly longer crosslinkers, such as Thermo Scientific DMP (Product # 21666, 21667), can also crosslink between subunits, but they may result in intermolecular coupling. Adjusting the reagent amount and protein concentration can control intermolecular crosslinking. Dilute protein solutions and high concentrations of crosslinker favor intramolecular crosslinking when homobifunctional crosslinkers are used.

For determination or confirmation of the three-dimensional structure, cleavable crosslinkers with increasing spacer arm lengths may be used to determine the distance between subunits. Experiments using crosslinkers with different reactive groups may indicate the locations of specific amino acids. Once conjugated, the proteins are subjected to two-dimensional electrophoresis. In the first dimension, the proteins are separated using non-reducing conditions and the molecular weights are recorded. Some subunits may not be crosslinked and will separate according to their individual molecular weights, while conjugated subunits will separate according to the combined size. The second dimension of the gel is then performed using conditions to cleave the crosslinked subunits. The individual molecular weights of the crosslinked subunits can be determined. Crosslinked subunits that were not reduced will produce a diagonal pattern, but the cleaved subunits will be off the diagonal. The molecular weights of the individual subunits should be compared with predetermined molecular weights of the protein subunits using reducing SDS-polyacrylamide gel electrophoresis.

Product #	Abbreviation	Chemical Name	Pkg. Size	M.W. (g/mole)	Spacer Arm (Å)	–NH_2 Amines
22295	AMAS	*N*-(α-Maleimidoacetoxy)-succinimide ester	50mg	252.18	4.4Å	**X**
21451	ANB-NOS	*N*-5-Azido-2-nitrobenzyloxy-succinimide	50mg	305.2	7.7Å	**X**
22331	BMB	1,4-*Bis*-Maleimidobutane	50mg	248.23	10.9Å	
22332	BMDB	1,4-*Bis*-Maleimmidyl-2,3-dihydroxy-butane	50mg	280.25	10.2Å	
22330	BMH	*Bis*-Maleimidohexane	50mg	276.29	16.1Å	
22323	BMOE	*Bis*-Maleimidoethane	50mg	220.18	8.0Å	
22297	BMPH	*N*-(β-Maleimidopropionic acid)hydrazide•TFA	50mg	297.19	8.1Å	
22298	BMPS	*N*-(β-Maleimidopropyloxy)succinimide ester	50mg	266.21	5.9Å	**X**
22336	$BM(PEG)_2$	1,8-*Bis*-Maleimidodiethylene-glycol	50mg	308.29	14.7Å	
22337	$BM(PEG)_3$	1,11-*Bis*-Maleimidotriethyleneglycol	50mg	352.34	17.8Å	
21580 **21585** **21586**	BS^3 (Sulfo-DSS)	*Bis* (sulfosuccinimidyl)suberate	50mg 8 x 2mg 1g	572.43	11.4Å	**X**
21610	BS^2G-d_0	*Bis* (sulfosuccinimidyl)glutarate-d_0	10mg	530.35	7.7Å	**X**
21615	BS^2G-d_4	*Bis* (sulfosuccinimidyl)2,2,4,4-glutarate-d_4	10mg	534.38	7.7Å	**X**
21590	BS^3-d_0	*Bis* (sulfosuccinimidyl)suberate-d_0	10mg	572.43	11.4Å	**X**
21595	BS^3-d_4	*Bis* (sulfosuccinimidyl)2,2,7,7-suberate-d_4	10mg	576.45	11.4Å	**X**
21581	$BS(PEG)_5$	*Bis* (NHS)PEG_5	100mg	532.5	21.7Å	**X**
21582	$BS(PEG)_9$	*Bis* (NHS)PEG_9	100mg	708.71	35.8Å	**X**
21600	BSOCOES	*Bis* (2-[succinimidoxycarbonyloxy]ethyl)sulfone	50mg	436.35	13Å	**X**
20320	DCC	*N,N*-Dicyclohexylcarbodiimide	100g	206.33	0Å	**X**
21525	DFDNB	1-5-Difluoro-2,4-dinitrobenzene	50mg	204.09	3Å	**X**
20660	DMA	Dimethyl adipimidate•2HCl	1g	245.15	8.6Å	**X**
21666 **21667**	DMP	Dimethyl pimelimidate•2HCl	50mg 1g	259.17	9.2Å	**X**
20700	DMS	Dimethyl suberimidate•2HCl	1g	273.20	11Å	**X**
20593	DSG	Disuccinimidyl glutarate	50mg	326.26	7.7Å	**X**
22585 **22586**	DSP	Dithiobis(succimidylpropionate) (Lomant's Reagent)	1g 50mg	404.42	12Å	**X**
21655 **21555** **21658**	DSS	Disuccinimidyl suberate	50mg 1g 8 x 2mg	368.35	11.4Å	**X**
20589	DST	Disuccinimidyl tartarate	50mg	344.24	6.4Å	**X**
20665	DTBP	Dimethyl 3,3'-dithiobis propionimidate•2HCl	1g	309.28	11.9Å	**X**
22335	DTME	Dithiobis-maleimidoethane	50mg	312.37	13.3Å	
21578	DTSSP (Sulfo-DSP)	3,3'-Dithiobis (sulfosuccinimidylpropionate)	50mg	608.51	12Å	**X**
77149 **22980** **22981**	EDC	1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride	10mg 5g 25g	191.70	0Å	**X**
21565	EGS	Ethylene glycol *bis* (succinimidylsuccinate)	1g	456.36	16.1Å	**X**
22306	EMCA	*N*-ε-Maleimidocaproic acid	1g	211.21	9.4Å	**X**

**Trifunctional crosslinking reagent; binds to Avidin, Streptavidin or NeutrAvidin™ Protein.*

*** Trifunctional crosslinking reagent.*

–SH Sulfhydryls	Carbohydrates	Nonselective (photo-reactive)	–COOH Carboxyls	–OH Hydroxyl	Phosphine	Hetero-bifunctional	Azides	Cleavable By
X						X		
		X				X		
X								
X								
X								
X								
X	X					X		
X						X		
X								
X								
								Base
			X			X		
								Thiols
								Periodate
								Thiols
X								Thiols
								Thiols
			X			X		
								Hydroxyl-amine
X						X		

Product #	Abbreviation	Chemical Name	Pkg. Size	M.W. (g/mole)	Spacer Arm (Å)	–NH_2 Amines
22106	EMCH	*N*-(ε-Maleimidocaproic acid)hydrazide	50mg	225.24	11.8Å	
22308	EMCS	*N*-(ε-Maleimidocaproyloxy)succinimide ester	50mg	308.29	9.4Å	**X**
22309	GMBS	*N*-(γ-Maleimidobutyryloxy)succinimide ester	50mg	280.23	7.3Å	**X**
22111	KMUH	*N*-(κ-Maleimidoundecanoic acid)hydrazide	50mg	295.38	19.0Å	
26168	LC-SDA	NHS-LC-Diazirine	50mg	338.36	12.5Å	**X**
22362	LC-SMCC	Succinimidyl 4-(*N*-maleimidomethyl) cyclohexane-1-carboxy-(6-amidocaproate)	50mg	447.48	16.2Å	**X**
21651	LC-SPDP	Succinimidyl 6-(3'-[2-pyridyl-dithio]propionamido)hexanoate	50mg	425.52	15.7Å	**X**
22610	L-Photo-Leucine		100mg	143.15	0Å	
22615	L-Photo-Methionine		100mg	157.17	0Å	
22311	MBS	*m*-Maleimidobenzoyl-*N*-hydroxysuccinimide ester	50mg	314.25	7.3Å	**X**
22305	MPBH	4-(4-*N*-Maleimidophenyl)-butyric acid hydrazide•HCl	50mg	309.75	17.9Å	
33093	Mts-Atf-Biotin**	2-[*N*2-(4-Azido-2,3,5,6-tetrafluorobenzoyl)-*N*6-(6-biotinamidocaproyl)-L-lysinyl]ethylmethanethiosulfate	5mg	839.95	Mts-Atf 11.1Å Mts-Biotin 29.3Å Atf-Biotin 30.7Å	
33083	Mts-Atf-LC-Biotin**	2-{*N*2-[*N*6-(4-Azido-2,3,5,6-tetrafluorobenzoyl)-*N*6-(6-biotinamidocaproyl)-L-lysinyl]}ethylmethanethiosulfate	5mg	953.11	Mts-Atf 21.8Å Mts-Biotin 29.3Å Atf-Biotin 35.2Å	
24500	NHS	*N*-Hydroxysuccinimide	25mg	115.09	NA	**X**
88902	NHS-Azide	*N*-hydroxysuccinimide ester ethane azide	10mg	198.14	2.5Å	**X**
26130	NHS-PEG_4-Azide	*N*-hydroxysuccinimide ester tetraoxapentadecane azide	100mg	388.37	18.9Å	**X**
26131	NHS-PEG_{12}-Azide	*N*-hydroxysuccinimide ester dodecaoxanonatriacontane azide	100mg	740.79	47.3Å	**X**
88900	NHS-Phosphine		10mg	461.40		**X**
22301	PDPH	3-(2-Pyridyldithio)propionylhydrazide	50mg	229.32	9.2Å	
26128	PEG_4-SPDP	2-pyridyldithiol-tetraoxatetradecane-*N*-hydroxysuccinimide	100mg	559.17	25.7Å	**X**
26129	PEG_{12}-SPDP	2-pyridyldithiol-tetraoxaoctatriacontane-*N*-hydroxysuccinimide	100mg	912.07	54.1Å	**X**
28100	PMPI	*N*-(*p*-Maleimidophenyl)isocyanate	50mg	214.18	8.7Å	
22339	SBAP	Succinimdyl 3-(bromoacetamido)propionate	50mg	307.10	6.2Å	**X**
26167	SDA	NHS-Diazirine	50mg	225.20	3.9Å	**X**
26169	SDAD	NHS-SS-Diazirine	50mg	388.46	13.5Å	**X**
22349	SIA	*N*-succinimidyl iodoacetate	50mg	283.02	1.5Å	**X**
22329	SIAB	*N*-Succinimidyl(4-iodoacetyl)aminobenzoate	50mg	402.14	10.6Å	**X**
22360	SMCC	Succinimidyl 4-(*N*-maleimido-methyl)cyclohexane-1-carboxylate	50mg	334.32	8.3Å	**X**
22102 **22103**	$SM[PEG]_2$	NHS-PEG_2-Maliemide	100mg 1g	425.39	17.6Å	**X**
22104 **22107**	$SM[PEG]_4$	NHS-PEG_4-Maliemide	100mg 1g	513.5	24.6Å	**X**
22105	$SM(PEG)_6$	NHS-PEG_6-Maleimide	100mg	601.6	32.5Å	**X**
22108	$SM[PEG]_8$	NHS-PEG_8-Maliemide	100mg	689.71	39.2Å	**X**
22112 **22113**	$SM[PEG]_{12}$	NHS-PEG_{12}-Maliemide	100mg 1g	865.92	53.4Å	**X**

**Trifunctional crosslinking reagent; binds to Avidin, Streptavidin or NeutrAvidin Protein.*

*** Trifunctional crosslinking reagent.*

–SH Sulfhydryls	Carbohydrates	Nonselective (photo-reactive)	–COOH Carboxyls	–OH Hydroxyl	Phosphine	Hetero-bifunctional	Azides	Cleavable By
X	X					X		
X						X		
X						X		
X	X					X		
		X				X		
X						X		
X		X				X		Thiols
		X						
X						X		
X	X					X		
X		X						Thiols
X		X						Thiols
					X			
					X			
					X			
							X	
X	X					X		Thiols
X								
X								
X				X		X		
X						X		
		X				X		
		X				X		Thiols
X						X		
X						X		
X						X		
X						X		
X						X		
X						X		
X						X		

Product #	Abbreviation	Chemical Name	Pkg. Size	M.W. (g/mole)	Spacer Arm (Å)	–NH_2 Amines
22114	$SM(PEG)_{24}$	$NHS\text{-}PEG_{24}$-Maleimide	102mg	1394.55	95.2Å	**X**
22416	SMPB	Succinimidyl 4-(*p*-maleimido-phenyl)butyrate	50mg	356.33	11.6Å	**X**
22363	SMPH	Succinimidyl-6-(β-maleimidopropionamido)hexanoate	50mg	379.36	14.2Å	**X**
21558	SMPT	4-Succinimidyloxycarbonyl-methyl-α-(2-pyridyldithio)toluene	50mg	388.46	20.0Å	**X**
23013	SPB	Succinimidyl-(4-psoralen-8-yloxy)butyrate	50mg	385.32	8.5-9.5Å	**X**
21857	SPDP	*N*-Succinimidyl 3-(2-pyridyldithio)propionate	50mg	312.37	6.8Å	**X**
21566	Sulfo-EGS	Ethylene glycol bis (sulfo-succinimidyl succinate)	50mg	660.45	16.1Å	**X**
22307	Sulfo-EMCS	*N*-(ε-Maleimidocaproyloxy)sulfosuccinimide ester	50mg	410.33	9.4Å	**X**
22324	Sulfo-GMBS	*N*-(γ-Maleimidobutryloxy)sulfosuccinimide ester	50mg	382.28	7.3Å	**X**
21111	Sulfo-KMUS	*N*-(κ-Maleimidoundecanoyloxy) sulfosuccinimide ester	50mg	485.47	16.3Å	**X**
26174	Sulfo-LC-SDA	Sulfo-NHS-LC-Diazirine	50mg	440.40	12.5Å	**X**
21650	Sulfo-LC-SPDP	Sulfosuccinimidyl 6-(3'-[2-pyridyl-dithio]propionamido)hexanoate	50mg	527.57	15.7Å	**X**
22312	Sulfo-MBS	*m*-Maleimidobenzoyl-***N***-hydroxysulfosuccinimide ester	50mg	416.30	7.3Å	**X**
24510 **24520** **24525**	Sulfo-NHS	*N*-Hydroxysuccinimide	500mg 8 x 2mg 5g	217.13	NA	**X**
88906	Sulfo-NHS-Phosphine		10mg	563.45		**X**
22589	Sulfo-SANPAH	Sulfosuccinimidyl 6-(4'-azido-2'-nitrophenylamino)hexanoate	50mg	492.40	18.2Å	**X**
33033 **33034**	Sulfo-SBED*	Sulfo-NHS-(2-6-[Biotinamido]-2-(*p*-azidobezamido)	10mg 8 x 1mg	879.98	Sulfo-NHS ester 13.7Å Phenyl azide 9.1Å Biotin 19.1Å	
26173	Sulfo-SDA	Sulfo-NHS-Diazirine	50mg	327.25	3.9Å	**X**
26175	Sulf-SDAD	Sulfo-NHS-SS-Diazirine	50mg	490.51	13.5Å	
22327	Sulfo-SIAB	Sulfosuccinimidyl(4-iodo-acetyl)aminobenzoate	50mg	504.19	10.6Å	**X**
22322 **22122** **22622**	Sulfo-SMCC	Sulfosuccinimidyl 4-(*N*-maleimido-methyl)cyclohexane-1-carboxylate	50mg 1g 8 x 2mg	436.37	8.3Å	**X**
22317	Sulfo-SMPB	Sulfosuccinimidyl 4-(*p*-maleimidophenyl)butyrate	50mg	458.38	11.6Å	**X**
33043	TMEA**	*Tris*-(2-Maleimidoethyl)amine (Trifunctional)	50mg	386.36	10.3Å	
33063	TSAT**	*Tris*-(succimimidyl aminotricetate) (Trifunctional)	50mg	482.36	4.2Å	**X**

**Trifunctional crosslinking reagent; binds to Avidin, Streptavidin or NeutrAvidin Protein.*

*** Trifunctional crosslinking reagent.*

–SH Sulfhydryls	Carbohydrates	Nonselective (photo-reactive)	–COOH Carboxyls	–OH Hydroxyl	Phosphine	Hetero-bifunctional	Azides	Cleavable By
X						X		
X						X		
X						X		Thiols
X						X		
		X				X		
X						X		Thiols
								Hydroxyl-amine
X						X		
X						X		
X						X		
		X				X		
X						X		Thiols
X						X		
							X	
		X				X		
X			X			X		Thiols
		X				X		
X						X		
X						X		
X						X		
X								

protein modification reagents

at a glance

Product #	Abbreviation	Chemical Name/Alternative Name	Pkg. Size	M.W. (g/mole)	Spacer Arm (Å)
35602	2-Mercaptoethanol	β-Mercaptoethanol	10 x 1mL	78.1	NA
20408	2-Mercaptoethylamine·HCl	β-Mercaptoethanol	6 x 6mg	78.1	NA
22101	AEDP	3-[(2-Aminoethyl)dithio]propionic acid•HCl	50mg	217.74	NA
23010	Aminoethylate Reagent	*N*-(Iodoethyl)trifluoroacetamide	1g	266.99	NA
44892	Sodium cyanoborohydride	AminoLink Reductant	2 x 1g	62.84	NA
26120 **26121**	$CA(PEG)_4$	Carboxyl-(4-ethyleneglycol) ethylamine	100mg 1g	265.3 265.3	18.1Å
26122 **26123**	$CA(PEG)_8$	Carboxyl-(8-ethyleneglycol) ethylamine	100mg 1g	441.5	33.6Å
26124 **26125**	$CA(PEG)_{12}$	Carboxyl-(12-ethyleneglycol) ethylamine	100mg 1g	617.7	46.8Å
26126 **26127**	$CA(PEG)_{24}$	Carboxyl-(24-ethyleneglycol) ethylamine	100mg 1g	1,146.4	89.8Å
26135	Carboxy-PEG-Lipoamide	45-(1,2-dithiolan-3-yl)-41-oxo-4,7,10,13,16,19,22,25,28,31,34,37-dodecaoxa-40-azapentatetracontan-1-oic acid	100mg	806.03	55.5Å
20907	Citraconic Anhydride	2-Methylmaleic anhydride	100g	112.08	NA
26133	$CT(PEG)_{12}$	1-Mercapto-3,6,9,12,15,18,21,24,27,30,33,36-dodecaoxanonatriacontan-39-oic acid	100mg	634.77	47.8Å
44889	Cysteine·HCl		5g	175.6	NA
20290 **20291**	DTT, Cleland's Reagent	Dithiothreitol	5g 48 x 7.7mg	154.3	NA
23031	Ethylenediamine·2HCl	Ethylenediamine dihydrochloride	10g	133.02	NA
24110	Guanidine·HCl	Guanidinium hydrochloride	500g	95.5	NA
24115	Guanidine·HCl Solution 8M	Guanidinium hydrochloride	200mL	95.54	NA
26103	Hydroxylamine·HCl	Hydroxylamine hydrochloride	25g	69.49	NA
35603	Iodoacetic Acid		500mg	185.95	NA
26110 **26111**	$MA(PEG)_4$	Methyl-(4-ethyleneglycol) amine	100mg 1g	207.3	15.5Å
26112 **26113**	$MA(PEG)_8$	Methyl-(8-ethyleneglycol) amine	100mg 1g	383.5	29.7Å
26114 **26115**	$MA(PEG)_{12}$	Methyl-(12-ethyleneglycol) amine	100mg 1g	559.7	43.9Å
26116 **26117**	$MA(PEG)_{24}$	Methyl-(24-ethyleneglycol) amine	100mg 1g	1,088.2	86.1Å
26134	$ML(PEG)_4$	5-(1,2-dithiolan-3-yl)-N-(2,5,8,11-tetraoxatridecan-13-yl) pentanamide	100mg	395.58	23.6Å
22711 **22712**	$MM(PEG)_{12}$	Methyl-PEG_{12}-maleimide	100mg 1g	710.8	51.9Å

Reactive Groups	Target Functional Group	Applications
Thiol	Disulfide bond reducing agent	Reducing agent
Thiol	Disulfide bond reducing agent	Reducing agent
Amine	*N*-Hydroxysuccinimide, oxidized carbohydrates	Addition of amine or carboxylic acid functional groups to protein or surface
Carboxylic acid	Amines via EDC	
Iodoacetyl	Reduced sulfhdryls	Protect sulfhdryls with trifluoroacetyl group
Cyanoborohydride	Reduce Schiff base to alkylamine bond	Reduce Schiff base to alkylamine linkage
Amine	*N*-hydroxysuccinimide, oxidized carbohydrates	Reversibly block primary amines
Carboxylic acid	Amines via EDC	
Amine	*N*-hydroxysuccinimide, oxidized carbohydrates	Pegylation of a protein or surface, terminating with a carboxylic acid or primary amine
Carboxylic acid	Amines via EDC	
Amine	*N*-hydroxysuccinimide, oxidized carbohydrates	Pegylation of a protein or surface, terminating with a carboxylic acid or primary amine
Carboxylic acid	Amines via EDC	
Amine	*N*-hydroxysuccinimide, oxidized carbohydrates	Pegylation of a protein or surface, terminating with a carboxylic acid or primary amine
Carboxylic acid	Amines via EDC	
Carboxylic acid	Amines via EDC	Pegylation of a gold or metal surface, terminating with a carboxylic acid
Bidentate thiol	Silver, gold and other metal surfaces	
	Amines	Reversibly block primary amines
Carboxylic acid	Amines via EDC	Pegylation of a gold or metal surface, terminating with a carboxylic acid
Thiol	Gold surfaces, maleimides, sulfhydryls, haloacetyls	
Thiol	Disulfide bond reducing agent	Reducing agent
Thiol	Disulfide bond reducing agent	Reducing agent
Amine	*N*-hydroxysuccinimide, oxidized carbohydrates	Addition of amines to protein or surface
NA	Denaturant	Protein denaturant
NA	Denaturant	Protein denaturant
NA	Cleaving Asn-Gly peptide bonds and deprotecting (exposing) sulfhydryls on SATA-modified molecules for conjugation.	Deprotecting SATA-modified molecules
Iodoacetyl	S-carboxymethylation of sulfhydryls (reduced cysteines)	S-carboxymethylation of sulfhydryls (reduced cysteines)
Amine	*N*-hydroxysuccinimide, oxidized carbohydrates	Pegylation of a protein, oxidized carbohydrate or surface, terminating with a methyl group
Amine	*N*-hydroxysuccinimide, oxidized carbohydrates	Pegylation of a protein, oxidized carbohydrate or surface, terminating with a methyl group
Amine	*N*-hydroxysuccinimide, oxidized carbohydrates	Pegylation of a protein, oxidized carbohydrate or surface, terminating with a methyl group
Amine	*N*-hydroxysuccinimide, oxidized carbohydrates	Pegylation of a protein, oxidized carbohydrate or surface, terminating with a methyl group
Bidentate thiol	Silver, gold and other metal surfaces	Pegylation of a gold or metal surface, terminating with a methyl group
Maleimide	Reduced sulfhydryls	Branched pegylation of a protein or surface, terminating with a methyl group

protein modification reagents

at a glance

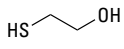
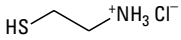
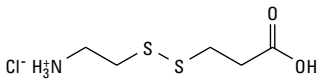
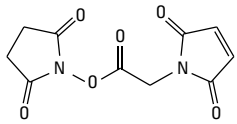
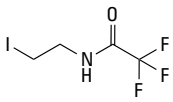
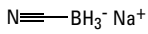
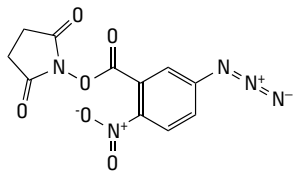
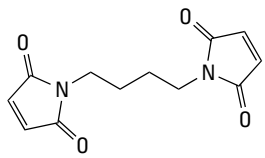
Product #	Abbreviation	Chemical Name/Alternative Name	Pkg. Size	M.W. (g/mole)	Spacer Arm (Å)
22713	$MM(PEG)_{24}$	Methyl-PEG_{24}-maleimide	100mg	1,239.4	95.3Å
23011	MMTS	Methyl methanethiosulfonate	200mg	126.2	NA
22341 **22342**	$MS(PEG)_4$	Methyl-PEG_4-NHS ester	100mg 1g	333.3	16.4Å
22509	$MS(PEG)_8$	Methyl-PEG_8-NHS ester	100mg	509.4	30.8Å
22685 **22686**	$MS(PEG)_{12}$	Methyl-PEG_{12}-NHS ester	100mg 1g	685.7	44.9Å
22687	$MS(PEG)_{24}$	Methyl-PEG_{24}-NHS ester	100mg	1,214.4	88.2Å
26132	$MT(PEG)_4$	2,5,8,11-Tetraoxatridecane-13-thiol	100mg	224.32	15.8Å
23030	NEM	*N*-ethylmaleimide	25g	125.13	NA
26099	$SAT(PEG)_4$	*N*-succinimidyl S-acetyl(thiotetraethylene glycol)	100mg	421.5	18.3Å
26102	SATA	*N*-succinimidyl S-acetylthioacetate	50mg	231.23	2.8Å
26100	SATP	*N*-hydroxysuccinimide ester	50mg	245.25	4.1Å
20504	Sodium meta-Periodate	Sodium meta-periodate	25g	213.89	NA
26777	Sulfo-NHS-Acetate	Sulfo-*N*-hydroxysulfosuccinimide acetate	100mg	259.17	NA
77720	TCEP Solution, Neutral pH	Disulfide bond reducing agent	5mL	250.2	NA
20490 **20491**	TCEP•HCl		1g 10g		
22299	TFCS	*N*-(ε-trifluoroacetylcaproyloxy)succinimide ester	50mg	324.25	7.7Å
22361	$TMM(PEG)_{12}$	(Methyl-PEG_{12})3-PEG_4-maleimide	100mg	2,360.8	77.6Å
22421 **22424**	$TMS(PEG)_{12}$	(Methyl-PEG_{12})3-PEG_4-NHS ester	100mg 1g	2,420.8	77.5Å
26101	Traut's Reagent	2-Iminothiolane	500mg	137.63	8.1Å
29700	Urea		1kg	60.06	NA

Reactive Groups	Target Functional Group	Applications
Maleimide	Reduced sulfhydryls	Branched pegylation of a protein or surface, terminating with a methyl group
	Reversibly blocks sulfhydryls (reduced cysteines)	Reversible sulfenylate thiol-containing molecules
N-hydroxysuccinimide ester	Primary amines	Pegylation of a protein or surface, terminating with a methyl group
N-hydroxysuccinimide ester	Primary amines	Pegylation of a protein or surface, terminating with a methyl group
N-hydroxysuccinimide ester	Primary amines	Pegylation of a protein or surface, terminating with a methyl group
N-hydroxysuccinimide ester	Primary amines	Pegylation of a protein or surface, terminating with a methyl group
Thiol	Gold surfaces, maleimides, sulfhydryls, haloacetyls	Pegylation of a protein or inert material surface, terminating with a methyl group
Maleimide	Reduced sulfhydryls	Irreversible blocking of sulfhydryl groups
N-hydroxysuccinimide ester	Primary amines	Modification of primary amines with protected sulfhydryl
Acetylated sulfhyfryl (protected)	Deprotectected with hydroxylamine for reactivity towards reduced sulfhydryls, haloacetyls or maleimides	
N-hydroxysuccinimide ester	Primary amines	Modify primary amines to contain a protected sulfhydryl group
N-hydroxysuccinimide ester	Primary amines	Modify primary amines to contain a protected sulfhydryl group
Periodate	Oxidize carbohydrates from reductive amination, diols	Oxidize carbohydrates for reductive amination
N-hydroxysuccinimide ester	Irreversibly block primary amines	Irreversibly block primary amines
Phosphine	Disulfide bond reducing agent	Reducing agent
		Reducing agent
N-hydroxysuccinimide ester	Primary amines	Protect and extend primary amines
Trifluoroacetyl-protected -NH_2	After deprotection, reactive towards *N*-hydroxysuccinimide, oxidized carbohydrates	
Maleimide	Reduced sulfhydryls	Branched pegylation of a protein or surface, terminating with a methyl group
N-hydroxysuccinimide ester	Primary amines	Branched pegylation of a protein or surface, terminating with a methyl group
Iminothiolane	Reacts with primary amines (e.g., lysine side chains) to add a small spacer arm (8.1Å) terminated by a free sulfhydryl group	Modify primary amines to contain a free sulfhydryl group
NA	Denaturant	Reducing agent

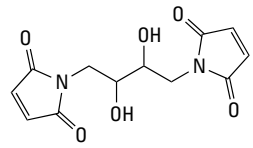
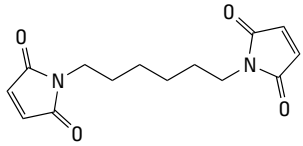
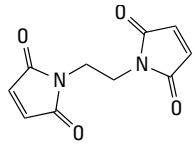
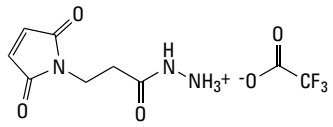
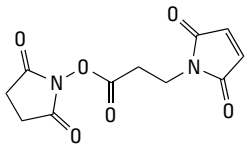
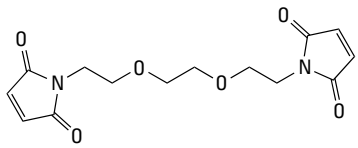
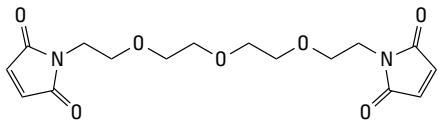
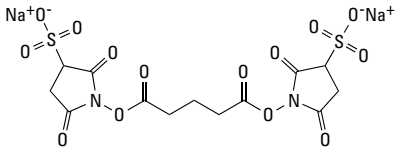
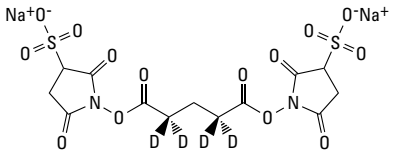
appendix 1

Structures and references

Chemical Structures

Product #	Product Name	Structure	Reference
35602	2-Mercaptoethanol **M.W. 78.1**		
20408	2-Mercaptoethylamine-HCl **M.W. 78.1**		• Yoshitake, S., *et al.* (1979). *Euro. J. Biochem.* **101(2)**, 395-399.
22101	AEDP **M.W. 217.74**		• Schnaar, R.L., *et al.* (1985). *Anal Biochem* **151**,268-81. • Sayre, L.M., *et al.* (1984). *J Med Chem* **27(10)**,1325-35.
22295	AMAS **M.W. 252.18** **Spacer Arm 4.4Å**		• Pech, M., *et al.* (2010). *J. Biol. Chem.* **285**, 19679-19687. • Tran, H. J., *et al.* (2005) *J. Biol. Chem.* **280**, 42423-42432. • Strang, C., *et al.* (2001). *J. Biol. Chem.* **276**, 28493-28502. • Chen, Z., *et al.* (2003). *J. Biol. Chem.* **278**, 48348-48356.
23010	Aminoethylate Reagent **M.W. 266.99**		• Schwartz, W.E., *et al.* (1980). *Anal Biochem* **106**,43-48.
44892	AminoLink Reductant **M.W. 62.84**		• Assad, F.F., *et al.* (2001). *J. Cell Biol.* **152**, 531-43. • Cheadle, C., *et al.* (1994). *J. Biol. Chem.* **269**, 24034-9. • Cofano, F. (1990). *J. Biol. Chem.* **265**, 4064-4071. • Czernik, P.J., and Hurlburt, B.K. (1994). *J. Biol. Chem.* **269**, 27869-75. • DeSilva, B.S. and Wilson, G.S. (1995). *J. Immunol. Meth.* **188**, 9-19. • Essler, M., *et al.* (2002). *Proc. Natl. Acad. Sci. USA.* **99**, 2252-7. • Fries, D.M., *et al.* (2003). *J. Biol. Chem.* **278**, 22901-7. • James, L.G., *et al.* (1994). *J. Biol. Chem.* **269**, 14182-90. • Kierszenbau, A.L., *et al.* (2003). *Mol. Biol. Cell* **14**, 4628-40. • Li, H. and Pajor, A.M. (2003). *Am. J. Physiol. Cell Physiol.* **285**, 1188-96. • Rivero-Lezcano, O.M., *et al.* (1994). *J. Biol. Chem.* **269**, 17363-6. • Rossig, L. *et al.* (2001). *Mol. Cell. Biol.* **21**, 5644-5657. • Zuk, P.A. and Elferink, L.A. (2000). *J. Biol. Chem.* **275**, 26754-64.
21451	ANB-NOS **M.W. 305.20** **Spacer Arm 7.7Å**		• Koulov, A. V., *et al.* (2010). *Mol. Biol. Cell* **21**, 871-884. • Górna, M. W., *et al.* (2010). *RNA* **16**, 553-562. • Adams, C. A., *et al.* (2004). *J. Biol. Chem.* **279**, 1376-1382. • Park, B. and Ahn, K. (2003). *J. Biol. Chem.* **278**, 14337-14345.
22331	BMB **M.W. 248.23** **Spacer Arm 10.9Å**		• Kida, Y., *et al.* (2007). *J. Cell Biol.* **179**, 1441-1452. • Auclair, J. R., *et al.* (2010). *Proc. Natl. Acad. Sci. USA* **107**, 21394-21399.

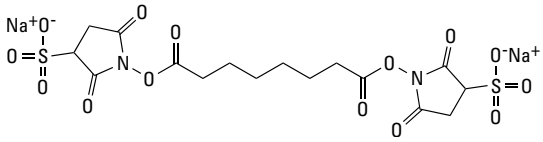
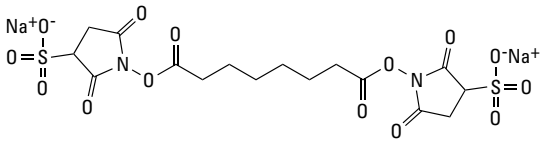
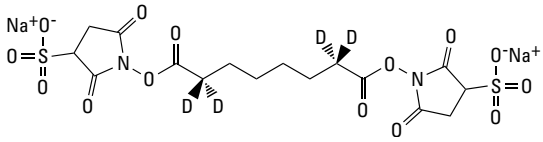
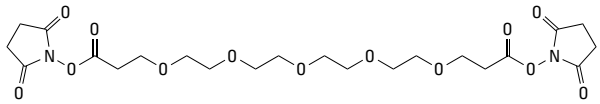
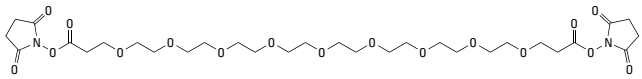
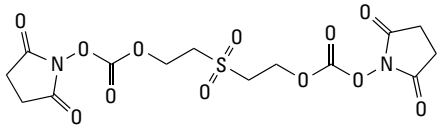
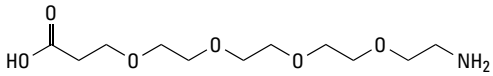
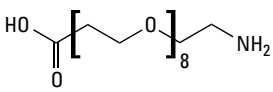
Chemical Structures

Product #	Product Name	Structure	Reference
22332	**BMDB** **M.W. 280.25** **Spacer Arm 10.2Å**		• Steiner, H., *et al.* (2008). *J. Biol. Chem.* **283**, 34677-34686. • Batra-Safferling, R., et al. (2006). *J. Biol. Chem.* **281**, 1449-1460. • Auclair, J. R., *et al.* (2010). *Proc. Natl. Acad. Sci. USA* **107**, 21394-21399. • Gavazzi, F., *et al.* (2007). *Plant Physiology* **145**, 933-945.
22330	**BMH** **M.W. 276.29** **Spacer Arm 16.1Å**		• Kida, Y., *et al.* (2007). *J. Cell Biol.* **179**, 1441-1452. • Rainer Beck, R., *et al.* (2008). *Proc. Natl. Acad. Sci. USA* **105**, 11731-11736. • Ahmad, R., *et al.* (2012). *J. Biol. Chem.* **287**, 20866-20875. • Gogada, R., *et al.* (2011). *J. Biol. Chem.* **286**, 28749-28760.
22323	**BMOE** **M.W. 220.18** **Spacer Arm 8.0Å**		• Geula, S., *et al.* (2012). *J. Biol. Chem.* **287**, 2179-2190. • Kida, Y., *et al.* (2007). *J. Cell Biol.* **179**, 1441-1452. • Auclair, J. R., *et al.* (2010). *Proc. Natl. Acad. Sci. USA* **107,** 21394-21399.
22297	**BMPH** **M.W. 297.19** **Spacer Arm 8.1Å**		• Özvegy-Laczka, C., *et al.* (2008). *J. Biol. Chem.* **283**, 26059-26070. • Ishmael, S. S., *et al.* (2006). *J. Biol. Chem.* **281**, 37404-37415.
22298	**BMPS** **M.W. 266.21** **Spacer Arm 5.9Å**		• Chen, Z., *et al.* (2003). *J. Biol. Chem.* **278**, 48348-48356.
22336	**BM(PEG)$_2$** **M.W. 308.29** **Spacer Arm 14.7Å**		• Auclair, J. R., *et al.* (2010). *Proc. Natl. Acad. Sci. USA* **107**, 21394-21399. • Kida, Y., *et al.* (2007). *J. Cell Biol.* **179**, 1441-1452.
22337	**BM(PEG)$_3$** **M.W. 352.34** **Spacer Arm 17.8Å**		• Auclair, J. R., *et al.* (2010). *Proc. Natl. Acad. Sci. USA* **107**, 21394-21399. • Kida, Y., *et al.* (2007). *J. Cell Biol.* **179**, 1441-1452.
21610	**BS^{2}G-d$_0$** **M.W. 530.35** **Spacer Arm 7.7Å**		
21615	**BS^{2}G-d$_4$** **M.W. 534.38** **Spacer Arm 7.7Å**		

appendix 1

Structures and references

Chemical Structures

Product #	Product Name	Structure	Reference
21580 21585 21586	BS³ (Sulfo-DSS) **M.W. 572.43** **Spacer Arm 11.4Å**		• Lay, F. T., *et al.* (2012). *J. Biol. Chem.* **287**, 19961-19972. • Kilkenny, M.L., *et al.* (2012). *J. Biol. Chem.* **287**, 23740-23747. • van Meeteren, L. A., *et al.* (2012). *J. Biol. Chem.* **287**, 18551-18561. • Richter, C., *et al.* (2012). *Mol. Cell. Biol.* **32**, 2515-2529. • Sachais, B. S., *et al.* (2012). *Blood* **119**, 5955-5962. • Ohgo, K., *et al.* (2012). *J. Biol. Chem.* **287**, 18201-18209. • Aryal, R. P., *et al.* (2012). *J. Biol. Chem.* **287**, 15317-15329. • Ohnishi, H., *et al.* (2012). *J. Biol. Chem.* **287**, 13371-13381.
21590	BS³-d_0 **M.W. 572.43** **Spacer Arm 11.4Å**		
21595	BS³-d_4 **M.W. 576.45** **Spacer Arm 11.4Å**		
21581	BS(PEG)$_5$ **M.W. 532.5** **Spacer Arm 21.7Å**		• Blank, K., *et al.* (2003). *Proc. Natl. Acad Sci. USA* **100**, 11356-11360. • Lay, F. T., *et al.* (2012). *J. Biol. Chem.* **287**, 19961-19972. • Petrie, R. J., *et al.* (2012). *J. Cell Biol.* **197**, 439-455.
21582	BS(PEG)$_9$ **M.W. 708.71** **Spacer Arm 35.8Å**		• Blank, K., *et al.* (2003). *Proc. Natl. Acad Sci. USA* **100**, 11356-11360. • Lay, F. T., *et al.* (2012). *J. Biol. Chem.* **287**, 19961-19972. • Petrie, R. J., *et al.* (2012). *J. Cell Biol.* **197**, 439-455.
21600	BSOCOES **M.W. 436.35** **Spacer Arm 13.0Å**		• Fenton, R. A., *et al.* (2007). *Am J Physiol Renal Physiol* **293**, F748-F760. • Grinberg, M., *et al.* (2005). *Mol. Cell. Biol.* **25**, 4579-4590
26120 26121	CA(PEG)$_4$ **M.W. 265.3** **Spacer Arm 18.1Å**		• Hermanson, G.T. (2008). *Bioconjugate Techniques*, Academic Press • Harris, J. M. and Zalipsky, S. Eds (1997). ACS Symposium Series, 680. • Harris, J. M. and Kozlowski, A. (2001). *J. Control Release* **72**, 217-224. • Veronese, F. and Harris, J.M. Eds. (2002). *Advanced Drug Delivery Review* **54(4)**, 453-609.
26122 26123	CA(PEG)$_8$ **M.W. 441.5** **Spacer Arm 33.6Å**		• Hermanson, G.T. (2008). *Bioconjugate Techniques*, Academic Press • Harris, J. M. and Zalipsky, S. Eds (1997). ACS Symposium Series, 680. • Harris, J. M. and Kozlowski, A. (2001). J. *Control Release* **72**, 217-224. • Veronese, F. and Harris, J.M. Eds. (2002). *Advanced Drug Delivery Review* **54(4)**, 453-609

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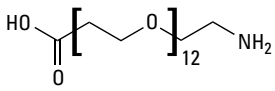
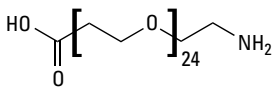
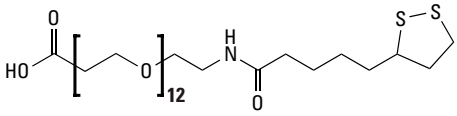
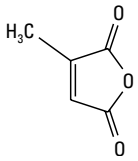
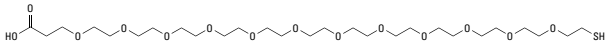
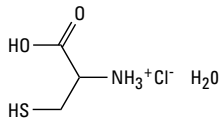
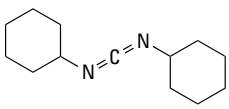
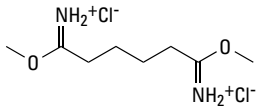
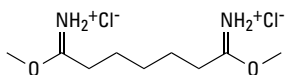
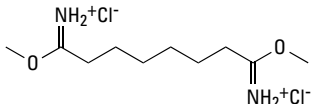
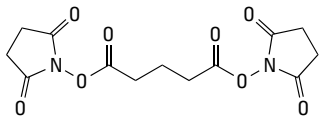
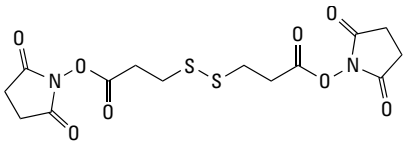
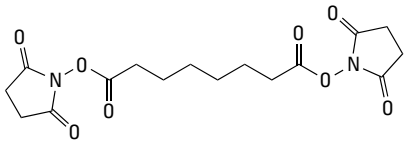
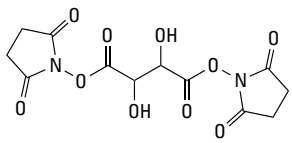
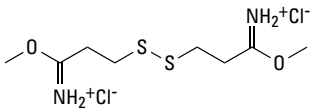
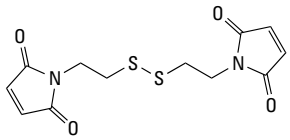
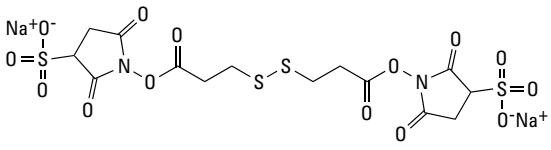
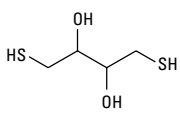
Product #	Product Name	Structure	Reference
26124 26125	CA(PEG)$_{12}$ **M.W. 617.1** **Spacer Arm 46.8Å**		• Hermanson, G.T. (2008). *Bioconjugate Techniques*, Academic Press • Harris, J. M. and Zalipsky, S. Eds (1997). Poly(ethylene glycol), Chemistry and Biological Applications, ACS Symposium Series, 680. • Harris, J. M. and Kozlowski, A. (2001). Improvements in protein PEGylation: pegylated interferons for treatment of hepatitis *C. J. Control Release* **72**, 217-224. • Veronese, F. and Harris, J.M. Eds. (2002). Peptide and protein PEGylation. *Advanced Drug Delivery Review* **54(4)**, 453-609.
26126 26127	CA(PEG)$_{24}$ **M.W. 1,146.4** **Spacer Arm 89.8Å**		• Hermanson, G.T. (2008). *Bioconjugate Techniques*, Academic Press • Harris, J. M. and Zalipsky, S. Eds (1997). ACS Symposium Series, 680. • Harris, J. M. and Kozlowski, A. (2001). *J. Control Release* **72**, 217-224. • Veronese, F. and Harris, J.M. Eds. (2002). *Advanced Drug Delivery Review* **54(4)**, 453-609.
26135	CL(PEG)$_{12}$ Carboxy-PEG$_{12}$-Lipoamide **M.W. 806.03** **Spacer Arm 55.5Å**		• Prime, K.L. and Whitesides, G.M. (1991). *Science* **252**, 1164. • Bentzen, E.L., *et al.* (2005). *Bioconjugate Chem* **16**, 1488-94. • Lin, P-C., *et al.* (2006). *Small* **2(4)**, 485-9. • Zheng, M., *et al.* (2003). *J Am Chem Soc* **125**, 7790-1. • Verma, A. and Rotello, V.M. (2005). *Chem Commun* **3**, 303-12. • Kidambi, S., *et al.* (2004). *J Am Chem Soc* **126**, 4697-03.
20907	Citraconic Anhydride **M.W. 112.08**		• Klapper, M.H. and Klotz, I.M. (1972). *Methods in Enzymology* **25**, 531-52.
26133	CT(PEG)$_{12}$ Carboxy-PEG$_{12}$-Thiol **M.W. 643.77** **Spacer Arm 47.8Å**		• Prime, K.L. and Whitesides, G.M. (1991). *Science* **252**, 1164. • Bentzen, E.L., *et al.* (2005). *Bioconjugate Chem* **16**, 1488-94. • Lin, P-C., *et al.* (2006). *Small* **2(4)**, 485-9. • Zheng, M., *et al.* (2003). *J Am Chem Soc* **125**, 7790-1. • Verma, A. and Rotello, V.M. (2005). *Chem Commun* **3**, 303-12. • Kidambi, S., *et al.* (2004). *J Am Chem Soc* **126**, 4697-03.
44889	Cysteine·HCl **M.W. 175.6**		
20320	DCC **M.W. 206.33** **Spacer Arm 0Å**		• Harney, A. S., *et al.* (2009). *Proc. Natl. Acad. Sci.* USA **106**, 13667-13672 • Sundaram, S., *et al.* (2009). *Mol. Cancer Ther.* **8**, 1655-1665. • Song, E-Q., *et al.* (2009). *Clin. Chem.* **55**, 955-963. • Chen, H., *et al.* (2008). *Proc. Natl. Acad. Sci. USA* **105**, 6596-6601.
21525	DFDNB **M.W. 204.09** **Spacer Arm 3.0Å**		• Geula, S., *et al.* (2012). *J. Biol. Chem.* **287**, 2179-2190. • Schulz, C., *et al.* (2011). *J. Cell Biol.* **195**, 643-656. • Sheridan, J. T., *et al.* (2011). *J. Biol. Chem.* **286**, 1381-1388. • Keinan, N., *et al.* (2010). *Mol. Cell. Biol.* **30**, 5698-5709. • Lucker, B. F., *et al.* (2010). *J. Biol. Chem.* **285**, 21508-21518. • Gruschke, S., *et al.* (2010). *J. Biol. Chem.* **285**, 19022-19028.
20660	DMA **M.W. 245.15** **Spacer Arm 8.6Å**		• Peng, L., *et al.* (2012). *Mol. Cell. Biol.* **32**, 2823-2836. • Fenton, R. A., *et al.* (2007). *Am J Physiol Renal Physiol* **293**, F748-F760. • Korczynska, J. E., *et al.* (2012). *Nucleic Acids Res.* **40**, 928-938. • Mizutani, A., *et al.* (2011). *J. Biol. Chem.* **286**, 29848-29860.
21666 21667	DMP **M.W. 259.17** **Spacer Arm 9.2Å**		• Fenton, R. A., *et al.* (2007). *Am J Physiol Renal Physiol* **293**, F748-F760. • Guergova-Kuras, M., *et al.* (2011). *Mol. Cell. Proteomics* **10**, M111.010298.

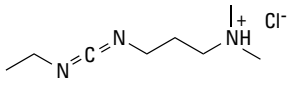
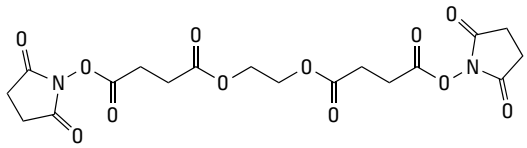
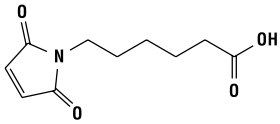
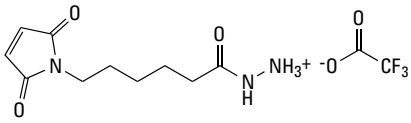
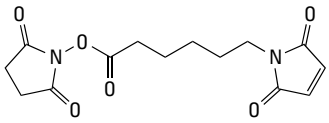
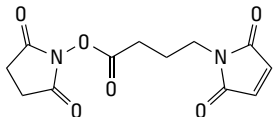
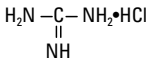
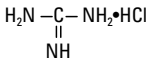
appendix 1

Structures and references

Chemical Structures

Product #	Product Name	Structure	Reference
20700	**DMS** **M.W. 273.20** **Spacer Arm 11.0Å**		• Korczynska, J. E., *et al.* (2012). *Nucleic Acids Res.* **40,** 928-938. • Sen, D., *et al.* (2012). *J. Biol. Chem.* **287**, 14545-14556. • Chang, T-W., *et al.* (2012). *J. Biol. Chem.* **287**, 418-428. • Guergova-Kuras, M., *et al.* (2011). *Mol. Cell. Proteomics* **10**, M111.010298. • Jha, B. K., *et al.* (2011). *Biol. Chem.* **286**, 26319-26326.
20593	**DSG** **M.W. 326.26** **Spacer Arm 7.7Å**		• Wang, Z., *et al.* (2012). *Nucleic Acids Res.* **40**, 4193-4202. • Akin, B. L. and Jones, L. R. (2012). *J. Biol. Chem.* **287**, 7582-7593. • Sen, N. and Snyder, S. H. (2011). *Proc. Natl. Acad. Sci. USA* **108,** 20178-20183. • Brasier, A. R., *et al.* (2011). *J. Virol.* **85**, 11752-11769. • Strang, C., *et al.* (2001). *J. Biol. Chem.* **276**, 28493-28502. • Mardaryev, A. N., *et al.* (2011). *Development* **138**, 4843-4852.
22585 **22586**	**DSP** **M.W. 404.42** **Spacer Arm 12.0Å**		• Larimore, J., *et al.* (2011). *Mol. Biol. Cell* **22**, 4854-4867. • Zlatic, S. A., *et al.* (2011). *Mol. Biol. Cell* **22**, 1699-1715. • Penmatsa, H., *et al.* (2010). *Mol. Biol. Cell* **21**, 1097-1110. • Gavazzi, F., *et al.* (2007). *Plant Physiology* **145**, 933-945. • Smith, A. L., *et al.* (2012). *Mol. Biol. Cell* **23**, 99-110. • Bue, C. A. and Barlowe, C. (2009). *J. Biol. Chem.* **284**, 24049-24060 • Steiner, H., *et al.* (2008). *J. Biol. Chem.* **283**, 34677-34686. • Denver, R. J. and Williamson, K. E. (2009). *Endocrinology* **150**, 3935-3943.
21655 **21555** **21658**	**DSS** **M.W. 368.35** **Spacer Arm 11.4Å**		• Jennebach, S., *et al.* (2012). Nucleic Acids Res. **40**, 5591-5601. • Ahmad, R., *et al.* (2012) J. Biol. Chem. **287**, 20866-20875. • van Meeteren, L. A., *et al.* (2012). *J. Biol. Chem.* **287**, 18551-18561. • Bofill-Cardona, E., *et al.* (2000). *J. Biol. Chem.* **275**, 32672-32680. • Yao, Y., *et al.* (2012). *Blood* **119**, 5037-5047. • Faye, A., *et al.* (2007). *J. Biol. Chem.* **282**, 26908-26916. • Sarkar, D. K., *et al.* (2012). *J. Biol. Chem.* **287**, 16734-16747.
20589	**DST** **M.W. 344.24** **Spacer Arm 6.4Å**		• Matsuda, M. *et al.* (2012). *Journal of Bioactive and Compatible Polymers* **27**, 31-44 • Yu, Y., *et al.* (2009). *Proc. Natl. Acad. Sci. USA* **106**, 11558-11563 • Alian, A., *et al.* (2009). *Proc. Natl. Acad. Sci. USA* **106**, 8192-8197. • Nakayama, K., *et al.* (2007). *Plant Physiology* **144**, 513-523.
20665	**DTBP** **M.W. 309.28** **Spacer Arm 11.9Å**		• Zhu, B., *et al.* (2012). *Mol. Cell. Biol.* **32**, 2065-2082. • Bhattacharya, M., *et al.* (2012). *Am J Physiol Lung Cell Mol Physiol* **303**, L12-L19. • Shi, Y., *et al.* (2011). *Mol. Biol. Cell* **22**, 4093-4107.
22335	**DTME** **M.W. 312.37** **Spacer Arm 13.3Å**		• Auclair, J. R., *et al.* (2010). *Proc. Natl. Acad. Sci. USA* **107**, 21394-21399. • Smith, A. L., *et al.* (2012). *Mol. Biol. Cell* **23**, 99-110. • Van Itallie, C. M., *et al.* (2011). *J. Biol. Chem.* **286**, 3442-3450. • Zhang, H., *et al.* (2010). *J. Biol. Chem.* **285**, 38324-38336. • Simonin, A. and Fuster, D. (2010). *J. Biol. Chem.* **285**, 38293-38303.
21578	**DTSSP (Sulfo-DSP)** **M.W. 608.51** **Spacer Arm 12.0Å**		• Hung, C-H., *et al.* (2012). *J. Biol. Chem.* **287**, 17398-17407. • Fenyk, S., *et al.* (2012). *J. Biol. Chem.* **287**, 4023-4032. • Boleij, A., *et al.* (2011). *Infect. Immun.* **79**, 4777-4783. • Li, H. and Pauza, C. D. (2011). *Blood* **118**, 5824-5831 • Liu, H., *et al.* (2011). *Proc. Natl. Acad. Sci. USA* **108**, 18536-18541.
20290 **20291**	**DTT, Cleland's Reagent** **M.W. 154.3**		• Zahler, W.L. and Cleland, W.W. (1964). *Biochemistry* **3**, 480-482.

Chemical Structures

Product #	Product Name	Structure	Reference
77149 22980 22981	**EDC** **M.W. 191.70** **Spacer Arm 0Å**		• Sperling, R. A. and Parak, W. J. (2010). *Phil Trans R Soc A* **368**, 1333-1383. • Bergfeld, A. K., *et al.* (2012). *J. Biol. Chem.* **287**, 28865-28881. • Kloetzel, P-M. and Janek, K. (2012). *Mol. Cell. Proteomics* **11**, 467-477. • Olsson, N., *et al.* (2012). *Mol. Cell. Proteomics* **11**, 342-354. • Adessi, C., *et al.* (2000). *Nucleic Acids Res.* **28**, e87. • Li, Z., *et al.* (2012). *Nucleic Acids Res.* **40**, 6787-6799. • Ido, K., *et al.* (2012). *J. Biol. Chem.* **287**, 26377-26387.
21565	**EGS** **M.W. 456.36** **Spacer Arm 16.1Å**		• Geula, S., *et al.* (2012). *J. Biol. Chem.* **287**, 2179-2190. • Mokry, M., *et al.* (2012). *Nucleic Acids Res.* **40**, 148-158. • Gogada, R., *et al.* (2011). *J. Biol. Chem.* **286**, 28749-28760. • Keinan, N., *et al.* (2010). *Mol. Cell. Biol.* **30**, 5698-5709. • Pourcet, B., *et al.* (2011). *Circ. Res.* **109**, 492-501.
22306	**EMCA** **M.W. 211.21** **Spacer Arm 9.4Å**		• Griffith, D.G., *et al.* (1981). *FEBS Lett.* **134**, 261-263.
22106	**EMCH** **M.W. 225.24** **Spacer Arm 11.8Å**		• Joyce, J., *et al.* (2006). *J. Biol. Chem.* **281**, 4831-4843. • Bao, X., *et al.* (2007). *Proc. Natl. Acad. Sci. USA* **104**, 4919-4924. • Ouertatani-Sakouhi, H., *et al.* (2010). *J. Biol. Chem.* **285**, 26581-26598.
22308	**EMCS** **M.W. 308.29** **Spacer Arm 9.4Å**		• Chen, Z., *et al.* (2003). *J. Biol. Chem.* **278**, 48348-48356. • Brandt, O., *et al.* (2003). *Nucleic Acids Res.* **31**, e119. • Chen, Z., *et al.* (2005). *J. Biol. Chem.* **280**, 10530-10539. • Akin, B. L. and Jones, L. R. (2012). *J. Biol. Chem.* **287**, 7582-7593. • Hamby, C. V., *et al.* (2005). *Clin. Diagn. Lab. Immunol.* **12**, 801-807. • Härmä, H., *et al.* (2000). *Clin. Chem.* **46**, 1755-1761. • Chen, Z., *et al.* (2007). *J. Biol. Chem.* **282**, 20968-20976.
23031	**Ethylenediamine·2HCl** **M.W. 133.02**		• Hermanson, G.T. (2008). *Bioconjugate Techniques, 2nd ed.*, Elsevier Inc., **114-118**, 125.
22309	**GMBS** **M.W. 280.23** **Spacer Arm 7.3Å**		• Fujiwara, K., *et al.* (1998). *J. Biochem.* **124**, 244-249. • Adessi, C., *et al.* (2000). *Nucleic Acids Res.* **28**, e87. • Chrisey, L. A., *et al.* (1996). *Nucleic Acids Res.* **24**, 3031-3039. • Lee, S-J., *et al.* (2011). *Blood* **117**, 5215-5223. • Gunning, A. P., *et al.* (2009). *FASEB J* **23**, 415-424. • Gunning, A. P., *et al.* (2008). *FASEB J* **22**, 2331-2339. • Lee, H-S., *et al.* (2010) *FASEB J* **24**, 2314-2324.
24110	**Guanidine·HCl** **M.W. 95.5**		• Tanaka, S., *et al.* (1985) *J. Biochem.* **97**, 1377-1384. • Wong, K.P., *et al.* (1971) *J. Biochem.* **40**, 459-464.
24115	**8M Guanidine·HCl Solution** **M.W. 95.54**		• Tanaka, S., *et al.* (1985) *J. Biochem.* **97**, 1377-1384. • Wong, K.P., *et al.* (1971) *J. Biochem.* **40**, 459-464.

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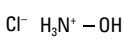
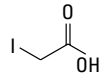
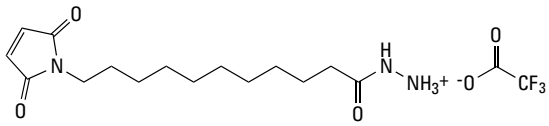
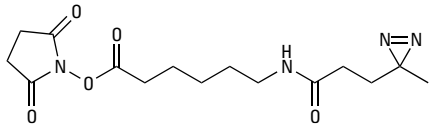
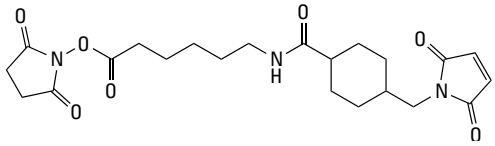
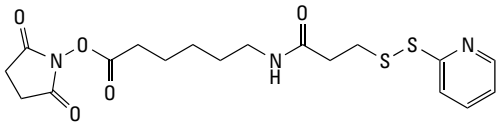
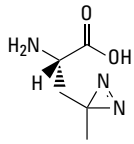
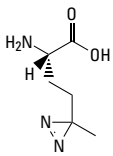
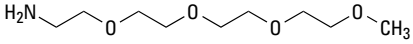
Product #	Product Name	Structure	Reference
26103	**Hydroxylamine-HCl** **M.W. 69.49**		• Abdella, P., *et al.* (1979). *Biochem Biophys Res Commun* **87(3)**, 732-42. • Duncan, R. J. S., *et al.* (1983). *Anal Biochem* **132**, 68-73.
35603	**Iodoacetic Acid** **M.W. 185.95**		• Hall, J., *et al.* (1989). *Biochem* 28:2568. • Hermason, G.T. (2008). *Bioconjugate Techniques, Second Edition.* Academic Press. pp. 109-111
22111	**KMUH** **M.W. 295.38** **Spacer Arm 19.0Å**		• Yang, W., *et al.* (2008). *Clin. Cancer Res.* **14,** 883-891. • Wu, G., *et al.* (2006). *Mol. Cancer Ther.* **5**, 52-59. • Yang, W., *et al.* (2006). *Clin. Cancer Res.* **12,** 3792-3802.
26168	**LC-SDA** **M.W. 338.36** **Spacer Arm 12.5Å**		
22362	**LC-SMCC** **M.W. 447.48** **Spacer Arm 16.2Å**		• Masuko, M. (2003). *Nucleic Acids Symp Ser* **3**, 145-146. • Mai Nguyen, M., *et al.* (2007). *Proc. Natl. Acad. Sci. USA* **104**, 19512-19517. • Ruffolo, S. C., and Shore, G. C. (2003). *J. Biol. Chem.* **278,** 25039-25045.
21651	**LC-SPDP** **M.W. 425.52** **Spacer Arm 15.7Å**		• Janganan, T. K., *et al.* (2011). *J. Biol. Chem.* **286**, 26900-26912. • Symmons, M. F., *et al.* (2009). *Proc. Natl. Acad. Sci. USA* **106**, 7173-7178. • Yuan, J. and Berg, H. C. (2008). *Proc. Natl. Acad. Sci. USA* **105,** 1182-1185. • Lobedanz, S., *et al.* (2007). *Proc. Natl. Acad. Sci. USA* **104**, 4612-4617.
22610	**L-Photo-Leucine** **M.W. 143.15**		• Zhao,W-Q., *et al.* (2010) *J. Biol. Chem.* **285**, 7619-7632.
22615	**L-Photo-Methionine** **M.W. 157.57**		• Weaver, M. S., *et al.* (2008). *J. Biol. Chem.* **283**, 22826-22837.
26110 **26111**	**MA(PEG)$_4$** **M.W. 207.3** **Spacer Arm 15.5Å**		• Hermanson, G.T. (2008). *Bioconjugate Techniques*, Academic Press • Harris, J. M. and Zalipsky, S. Eds (1997). ACS Symposium Series, 680. • Harris, J. M. and Kozlowski, A. (2001). *J. Control Release* **72**, 217-224. • Veronese, F. and Harris, J.M. Eds. (2002). *Advanced Drug Delivery Review* **54(4)**, 453-609.

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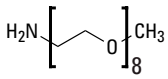
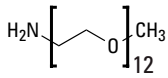
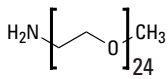
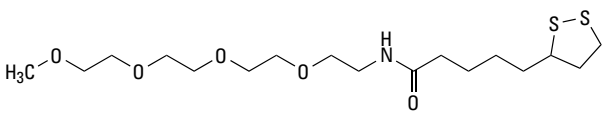
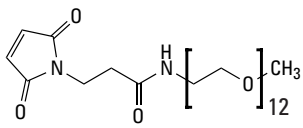
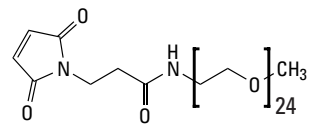
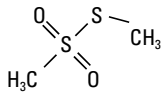
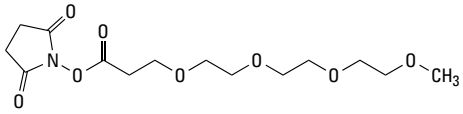
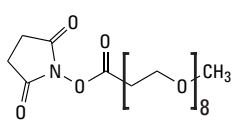
26112 26113	MA(PEG)$_8$ **M.W. 383.5** **Spacer Arm 29.7Å**		• Hermanson, G.T. (2008). *Bioconjugate Techniques*, Academic Press • Harris, J. M. and Zalipsky, S. Eds (1997). ACS Symposium Series, 680. • Harris, J. M. and Kozlowski, A. (2001). *J. Control Release* **72**, 217-224. • Veronese, F. and Harris, J.M. Eds. (2002). *Advanced Drug Delivery Review* **54(4)**, 453-609.
26114 26115	MA(PEG)$_{12}$ **M.W. 559.7** **Spacer Arm 43.9Å**		• Hermanson, G.T. (2008). *Bioconjugate Techniques*, Academic Press • Harris, J. M. and Zalipsky, S. Eds (1997). ACS Symposium Series, 680. • Harris, J. M. and Kozlowski, A. (2001). *J. Control Release* **72**, 217-224. • Veronese, F. and Harris, J.M. Eds. (2002). *Advanced Drug Delivery Review* **54(4)**, 453-609.
26116 26117	MA(PEG)$_{24}$ **M.W. 1,088.2** **Spacer Arm 86.1Å**		• Hermanson, G.T. (2008). *Bioconjugate Techniques*, Academic Press • Harris, J. M. and Zalipsky, S. Eds (1997). ACS Symposium Series, 680. • Harris, J. M. and Kozlowski, A. (2001). *J. Control Release* **72**, 217-224. • Veronese, F. and Harris, J.M. Eds. (2002). *Advanced Drug Delivery Review* **54(4)**, 453-609.
26134	ML(PEG)$_4$ Methyl-PEG$_4$-Lipoamide **M.W. 359.58** **Spacer Arm 23.6Å**		• Prime, K.L. and Whitesides, G.M. (1991). *Science* **252**, 1164. • Bentzen, E.L., *et al.* (2005). *Bioconjugate Chem* **16**,1488-94. • Lin, P-C., *et al.* (2006). *Small* **2(4)**, 485-9. • Zheng, M., *et al.* (2003). *J Am Chem Soc* **125**, 7790-1. • Verma, A. and Rotello, V.M. (2005). *Chem Commun* **3**, 303-12. • Kidambi, S., *et al.* (2004). *J Am Chem Soc* **126**, 4697-03.
22711 22712	MM(PEG)$_{12}$ **M.W. 710.8** **Spacer Arm 51.9Å**		• Hermanson, G.T. (2008). *Bioconjugate Techniques*, Academic Press. • Harris, J. M. and Zalipsky, S. Eds (1997). ACS Symposium Series, 680. • Harris, J. M. and Kozlowski, A. (2001). I C. *J. Control Release* **72**, 217-224. • Veronese, F. and Harris, J.M. Eds. (2002). *Advanced Drug Delivery Review* **54(4)**, 453-609.
22713	MM(PEG)$_{24}$ **M.W. 1,239.4** **Spacer Arm 95.3Å**		• Hermanson, G.T. (2008). *Bioconjugate Techniques*, Academic Press. • Harris, J. M. and Zalipsky, S. Eds (1997). ACS Symposium Series, 680. • Harris, J. M. and Kozlowski, A. (2001). I C. *J. Control Release* **72**, 217-224. • Veronese, F. and Harris, J.M. Eds. (2002). *Advanced Drug Delivery Review* **54(4)**, 453-609.
23011	MMTS **M.W. 126.2**		• Kirley, T.L. (1989). *Anal Biochem* **180**, 231. • Smith, D.J., *et al.* (1975). *Biochemistry* **14**, 766.
22341 22342	MS(PEG)$_4$ **M.W. 333.3** **Spacer Arm 16.4Å**		• Hermanson, G.T. (2008). *Bioconjugate Techniques*, Academic Press • Harris, J. M. and Zalipsky, S. Eds (1997). ACS Symposium Series, 680. • Harris, J. M. and Kozlowski, A. (2001). *J. Control Release* **72**, 217-224. • Veronese, F. and Harris, J.M. Eds. (2002). *Advanced Drug Delivery Review* **54(4)**, 453-609.
22509	MS(PEG)$_8$ **M.W. 509.4** **Spacer Arm 30.8Å**		• Hermanson, G.T. (2008). *Bioconjugate Techniques*, Academic Press • Harris, J. M. and Zalipsky, S. Eds (1997). ACS Symposium Series, 680. • Harris, J. M. and Kozlowski, A. (2001). *J. Control Release* **72**, 217-224. • Veronese, F. and Harris, J.M. Eds. (2002). *Advanced Drug Delivery Review* **54(4)**, 453-609.

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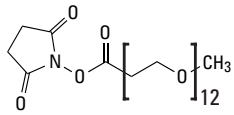
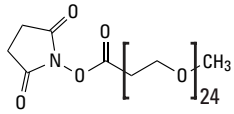
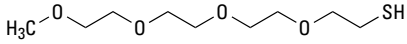
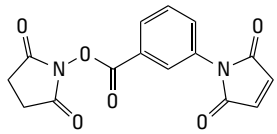
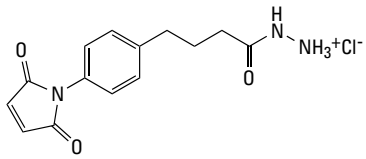
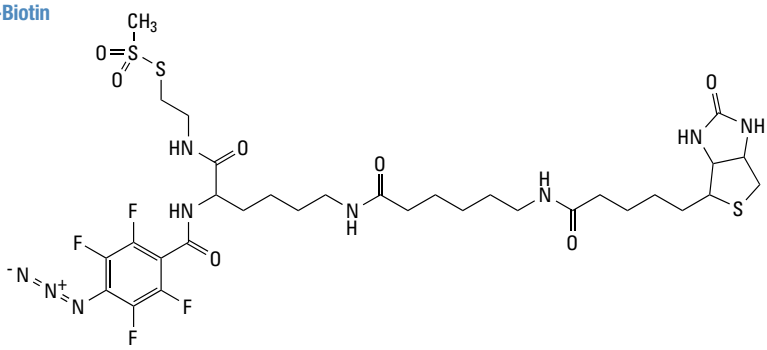
22685 22686	MS(PEG)$_{12}$ **M.W. 685.7** **Spacer Arm 44.9Å**		• Hermanson, G.T. (2008). *Bioconjugate Techniques*, Academic Press • Harris, J. M. and Zalipsky, S. Eds (1997). ACS Symposium Series, 680. • Harris, J. M. and Kozlowski, A. (2001). *J. Control Release* **72**, 217-224. • Veronese, F. and Harris, J.M. Eds. (2002). *Advanced Drug Delivery Review* **54(4)**, 453-609.
22687	MS(PEG)$_{24}$ **M.W. 1,214.4** **Spacer Arm 88.2Å**		• Hermanson, G.T. (2008). *Bioconjugate Techniques*, Academic Press. • Harris, J. M. and Zalipsky, S. Eds (1997). ACS Symposium Series, 680. • Harris, J. M. and Kozlowski, A. (2001). C. *J. Control Release* **72**, 217-224. • Veronese, F. and Harris, J.M. Eds. (2002). *Advanced Drug Delivery Review* **54(4)**, 453-609.
26132	MT(PEG)$_4$ Methyl-PEG$_4$-Thiol **M.W. 224.32** **Spacer Arm 15.8Å**		• Prime, K.L. and Whitesides, G.M. (1991). Self-assembled organic monolayers: model systems for studying absorption of proteins at surfaces. *Science* **252**, 1164. • Bentzen, E.L., *et al.* (2005). Surface modification to reduce non-specific binding of quantum dots in live cell assays. *Bioconjugate Chem* **16**, 1488-94. • Lin, P-C., *et al.* (2006). Ethylene glycol-protected magnetic nanoparticles for a multiplexed immunoassay in human plasma. *Small* **2(4)**, 485-9. • Zheng, M., *et al.* (2003). Ethylene glycol monolayer protected nanoparticles for eliminating nonspecific binding with biological molecules. *J Am Chem Soc* **125**, 7790-1. • Verma, A. and Rotello, V.M. (2005). Surface recognition of biomacromolecules using nanoparticle receptors. *Chem Commun* **3**, 303-12. • Kidambi, S., *et al.* (2004). Selective depositions on polyelectrolyte multilayers: self-assembled monolayers of m-dPEG acid as molecular template. *J Am Chem Soc* **126**, 4697-03.
22311	MBS **M.W. 314.25** **Spacer Arm 7.3Å**		• Adessi, C., *et al.* (2000). *Nucleic Acids Res.* **28**, e87. • Chrisey, L. A., *et al.* (1996). *Nucleic Acids Res.* **24**, 3031-3039. • Chen, Z., *et al.* (2003). *J. Biol. Chem.* **278**, 48348-48356.
22305	MPBH **M.W. 309.75** **Spacer Arm 17.9Å**		• Chamow, S. M., *et al.* (1992). *J. Biol. Chem.* **267**, 15916-15922. • Gunning, A. P., *et al.* (2009). *FASEB J* **23**, 415-424. • Gunning, A. P., *et al.* (2008). *FASEB J* **22**, 2331-2339.
33093	Mts-Atf-Biotin **Mww**		• Layer, G., *et al.* (2007). *J. Biol. Chem.* **282**, 13342-13350. • Padrick, S. B., *et al.* (2011). *Proc. Natl. Acad Sci. USA* **108**, E472-E479. • Wätzlich, D., *et al.* (2009). *J. Biol. Chem.* **284**, 15530-15540.

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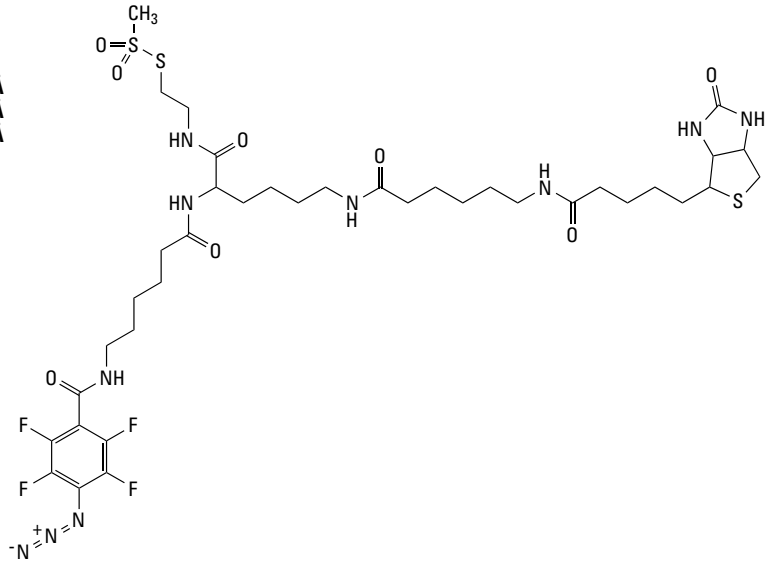
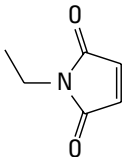
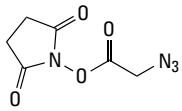
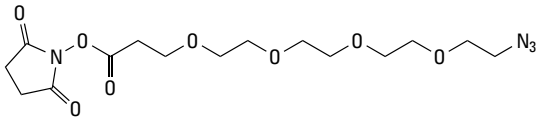
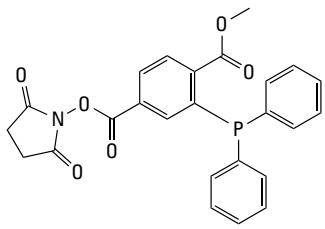
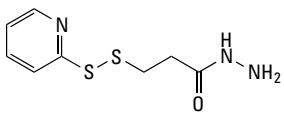
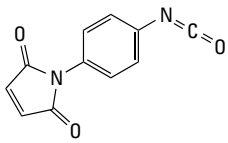
Product #	Product Name	Structure	Reference
33083	**Mts-Atf-LC-Biotin** **M.W. 953.11** **Spacer Arms** **Mts-Atf 21.8Å** **Mts-Biotin 29.3Å** **Atf-Biotin 35.2Å**		
23030	**N-Ethylmaleimide (NEM)** **M.W. 125.13**		• Partis, M.D., *et al.* (1983). *J Prot Chem* **2(3)**, 263-77.
88902	**NHS-Azide** **M.W. 198.54**		
26130 **26131**	**NHS-PEG$_4$-Azide** **M.W. 388.37** **Spacer Arm 18.9Å**		
88900	**NHS-Phosphine** **M.W. 461.4**		• Jumper, C. C. and Schriemer, D. C. (2011). *Anal. Chem.* **83(8)**, 2913–2920.
22301	**PDPH** **M.W. 229.32** **Spacer Arm 9.2Å**		• Atkinson, S. F., *et al.* (2001). *J. Biol. Chem.* **276**, 27930-27935. • Bäckman, C., *et al.* (1996). *J. Neurosci.* **16**, 5437-5442. • Govindan, S. V., *et al.* (1995). *Cancer Res.* **55**, 5721s-5725s.
28100	**PMPI** **M.W. 214.18** **Spacer Arm 8.7Å**		• Özvegy-Laczka, C., *et al.* (2008). *J. Biol. Chem.* **283**, 26059-26070. • Shen, G., *et al.* (2004). *Nucleic Acids Res.* **32,** 5973-5980 • Chirayil, S., *et al.* (2009). *Nucleic Acids Res.* **37**, 5486-5497. • Pourmand, N., *et al.* (2006). *Proc. Natl. Acad. Sci. USA* **103**, 6466-6470.

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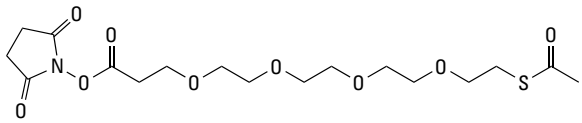
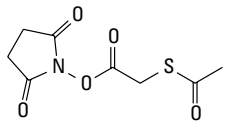
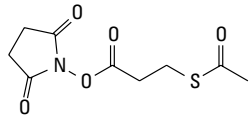
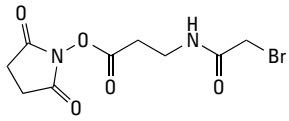
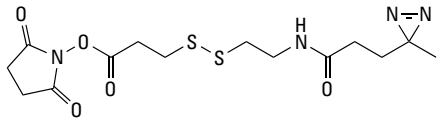
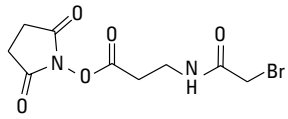
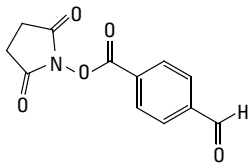
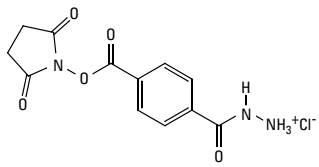
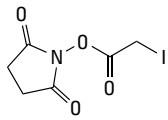
Structures and references

Chemical Structures

Product #	Product Name	Structure	Reference
26099	SAT(PEG)$_4$ **M.W. 421.5** **Spacer Arm 18.3Å**		• Hermanson, G.T. (2008). *Bioconjugate Techniques*, Academic Press.
26102	SATA **M.W. 231.23** **Spacer Arm 2.8Å**		• Duncan, R.J.S., *et al.* (1983). *Anal Biochem* **132**, 68-73.
26100	SATP **M.W. 245.25** **Spacer Arm 4.1Å**		• Duncan, R.J.S., *et al.* (1983). *Anal Biochem* **132**, 68-73. • Fuji, N., *et al.* (1985). *Chem Pharm Bull* **33**, 362-7.
22339	SBAP **M.W. 307.10** **Spacer Arm 6.2Å**		
26167	SDA **M.W. 388.46** **Spacer Arm 13.5Å**		
26169	SDAD **M.W. 388.46** **Spacer Arm 13.5Å**		• Inman, J.K., *et al.* (1991). *Bioconjug. Chem.* **2**, 458-463.
22419	SFB **M.W. 247.20** **Spacer Arm 5.8Å**		
22411	SHTH **M.W. 311.68** **Spacer Arm 7.9Å**		
22349	SIA **M.W. 283.02** **Spacer Arm 1.5Å**		• Chen,Y-R., *et al.* (2004). *J. Biol. Chem.* **279**, 18054-18062. • Strang, C., *et al.* (2001). *J. Biol. Chem.* **276**, 28493-28502.

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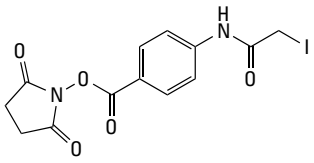
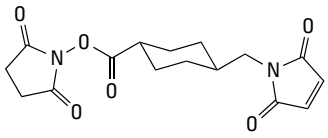
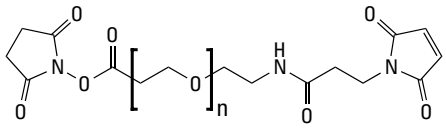
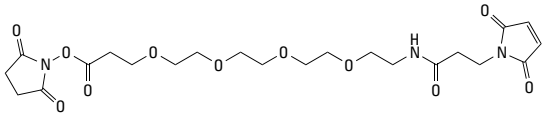
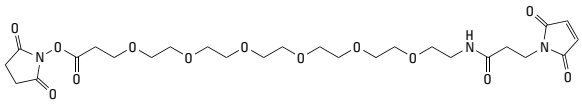
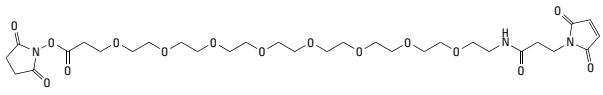
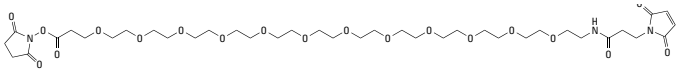
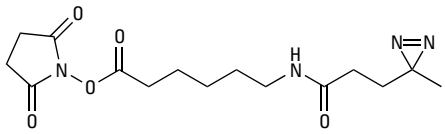
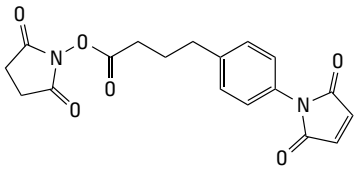
Product #	Product Name	Structure	Reference
22329	SIAB M.W. 402.14 Spacer Arm 10.6Å		• Stan, T., *et al.* (2003). *Mol. Cell. Biol.* **23**, 2239-2250. • Andreadis, J. D. and Chrisey, L. A. (2000). *Nucleic Acids Res.* **28**, e5. • Tang, K., *et al.* (1999). *Proc. Natl. Acad. Sci. USA* **96**, 10016-10020. • Weng, G., *et al.* (1996). *J. Biol. Chem.* **271**, 26445-26448. • Chrisey, L. A., *et al.* (1996). *Nucleic Acids Res.* **24**, 3031-3039.
22360	SMCC M.W. 334.32 Spacer Arm 8.3Å		• Kovtun, Y. V., *et al.* (2010). *Cancer Res.* **70**, 2528-2537. • Chrisey, L. A., *et al.* (1996). *Nucleic Acids Res.* **24**, 3031-3039. • Chen, Z., *et al.* (2003). *J. Biol. Chem.* **278**, 48348-48356. • Adessi, C., *et al.* (2000). *Nucleic Acids Res.* **28**, e87. • Hatakeyama, S., *et al.* (2011). *Proc. Natl. Acad. Sci. USA* **108**, 19587-19592. • Sperling, R. A. and Parak, W. (2010). *J. Phil Trans R Soc A* **368**, 1333-1383. • Obara, K., *et al.* (2001). *Nucleic Acids Symp Ser* **1**, 217-218.
22102 22103	SM(PEG)$_2$ M.W. 425.39 Spacer Arm 17.6Å		
22104 22107	SM(PEG)$_4$ M.W. 513.5 Spacer Arm 24.6Å		
22105	SM(PEG)$_6$ M.W. 601.6 Spacer Arm 32.5Å		
22108	SM(PEG)$_8$ M.W.689.71 Spacer Arm 39.2Å		
22112 22113	SM(PEG)$_{12}$ M.W. 865.92 Spacer Arm 53.4Å		
22114	SM(PEG)$_{24}$ M.W. 1394.55 Spacer Arm 95.2Å		
22416	SMPB M.W. 356.33 Spacer Arm 11.6Å		• Meng, F. and Sachs, F. (2011). *J. Cell Sci.* **124**, 261-269. • Karumuthil-Melethil, S., *et al.* (2010). *J. Immunol.* **184**, 6695-6708. • Chrisey, L. A., *et al.* (1996). *Nucleic Acids Res.* **24**, 3031-3039. • Goldoni, S., *et al.* (2009). *J. Cell Biol.* **185**, 743-754.

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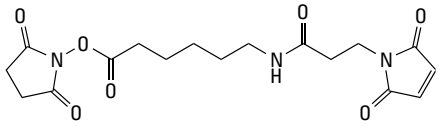
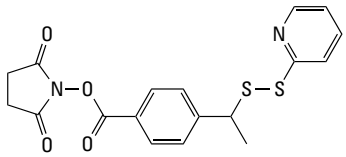
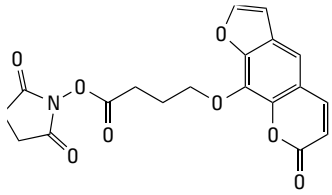
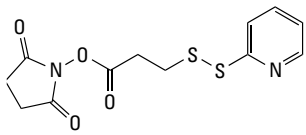
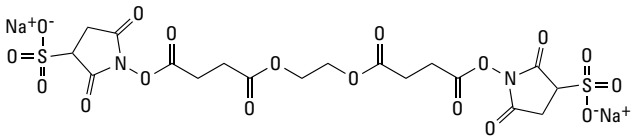
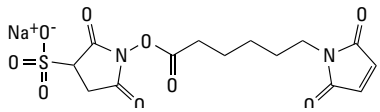
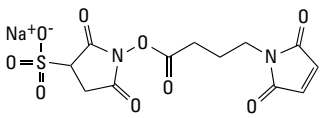
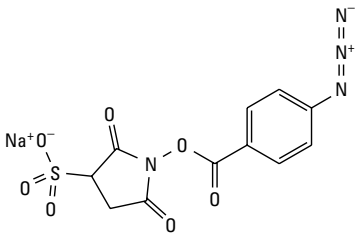
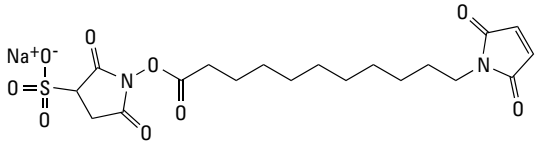
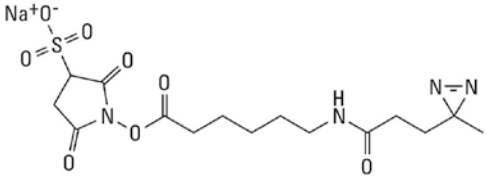
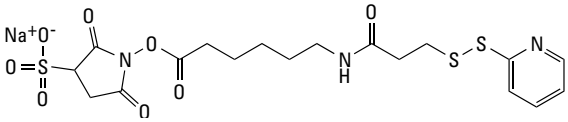
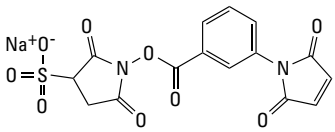
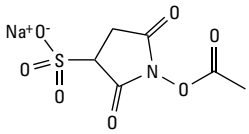
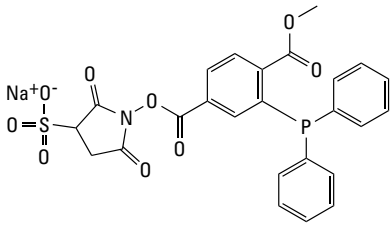
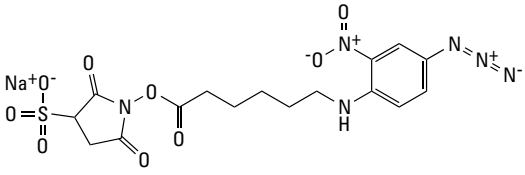
Structures and references

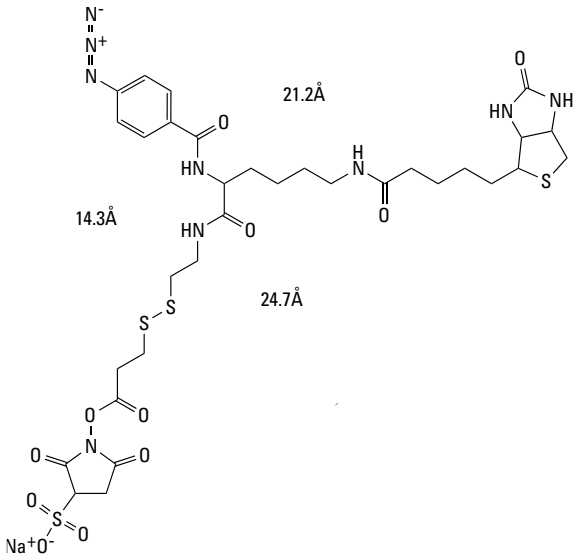
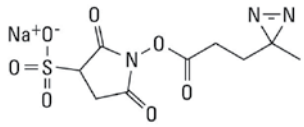
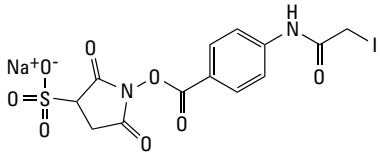
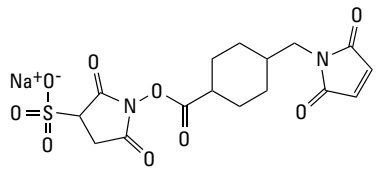
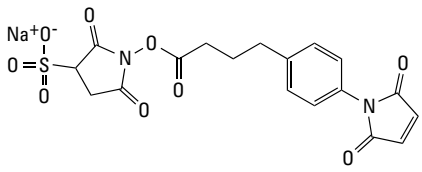
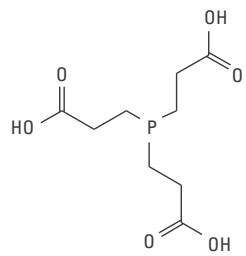
Chemical Structures

Product #	Product Name	Structure	Reference
22363	**SMPH** **M.W. 379.36** **Spacer Arm 14.2Å**		• Chen, Z., *et al.* (2003). *J. Biol. Chem.* **278**, 48348-48356. • Schmitz, N., *et al.* (2009). *J. Exp. Med.* **206**, 1941-1955.
21558	**SMPT** **M.W. 388.46** **Spacer Arm 20.0Å**		• Austin, C. D., *et al.* (2005). *Proc. Natl. Acad. Sci. USA* **102**, 17987-17992. • Solomon, S. R., *et al.* (2005). *Blood* **106**, 1123-1129. • Windt, W., *et al.* (2004). *Journal of Renin-Angiotensin-Aldosterone System* **5**, 197-202. • Ding, B-S., *et al.* (2003). *Circulation* **108**, 2892-2898.
20504	**Sodium Meta-Periodate** **M.W. 213.89**		• Hermanson, G.T. (2008). *Bioconjugate Techniques, Second Edition,* Academic Press. pp. 130-131.
23013	**SPB** **M.W. 385.32** **Spacer Arm 8.6Å**		
21857	**SPDP** **M.W. 312.37** **Spacer Arm 6.8Å**		• Karumuthil-Melethil, S. *et al.* (2010). *J. Immunol.* **184,** 6695-6708. • You, H. *et al.* (2012). *Nucleic Acids Res.* **10**.1093/nar/gks651. • Pallaoro, A., *et al.* (2011). *Proc. Natl. Acad. Sci. USA* **108**, 16559-16564. • Lobedanz, S., *et al.* (2007). *Proc. Natl. Acad. Sci. USA* **104,** 4612-4617. • Janganan, T. K., *et al.* (2011). *J. Biol. Chem.* **286**, 26900-26912. • Zhou, H., *et al.* (2011). *Mol. Cancer Ther.* **10**, 1276-1288.
21566	**Sulfo-EGS** **M.W. 660.45** **Spacer Arm 16.1Å**		• Xayarath, B., *et al.* (2011). *Microbiology* **157**, 3138-3149. • Xing, Y., *et al.* (2004). *J. Biol. Chem.* **279**, 30662-30669. • Özvegy-Laczka, C., *et al.* (2008). *J. Biol. Chem.* **283**, 26059-26070. • Deiss, K., *et al.* (2012). *J. Biol. Chem.* **287**, 23407-23417. • Kim, J., *et al.* (2011). *J. Virol.* **85**, 8116-8132. • Séverin, S., *et al.* (2011). *J. Biol. Chem.* **286**, 4107-4116 • Jansma, A. L., *et al.* (2010). *J. Biol. Chem.* **285**, 14424-14437.
22307	**Sulfo-EMCS** **M.W. 410.33** **Spacer Arm 9.4Å**		• Anderson, J. P., *et al.* (2006). *J. Biol. Chem.* **281**, 29739-29752. • Fixe, F., *et al.* (2004). *Nucleic Acids Res.* **32**, e70. • Fixe, F., *et al.* (2004). *Nucleic Acids Res.* **32**, e9.
22324	**Sulfo-GMBS** **M.W. 382.28** **Spacer Arm 7.3Å**		• Wang, Q. S., *et al.* (2011). *RNA* **17**, 469-477. • Adessi, C., *et al.* (2000). *Nucleic Acids Res.* **28**, e87. • Faye, A., *et al.* (2007). *J. Biol. Chem.* **282**, 26908-26916. • Weber, A. N. R., *et al.* (2005). *J. Biol. Chem.* **280**, 22793-22799. • Liu, S-H., *et al.* (2006). *Mol. Cell. Proteomics* **5**, 1019-1032.

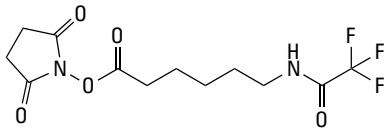
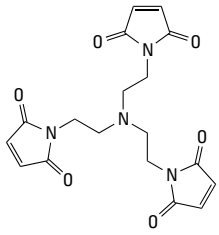
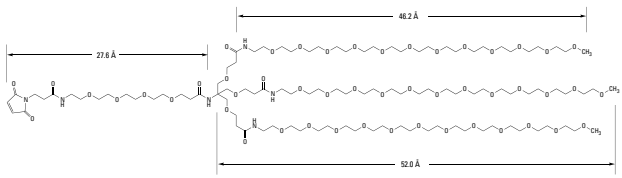
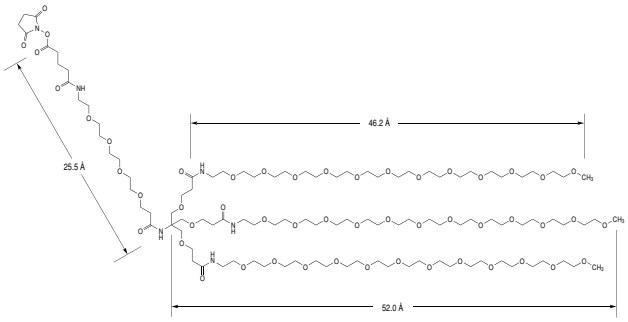
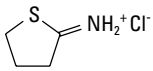
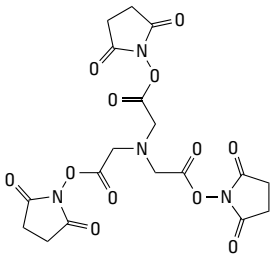
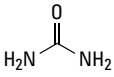
Chemical Structures

Product #	Product Name	Structure	Reference
21563	Sulfo-HSAB **M.W. 362.25** **Spacer Arm 9.0Å**		
21111	Sulfo-KMUS **M.W. 480.47** **Spacer Arm 16.3Å**		• DeCory, T. R., *et al.* (2005). *Appl. Envir. Microbiol.* **71**, 1856-1864. • Faye, A., *et al.* (2007). *J. Biol. Chem.* **282**, 26908-26916. • Weber, A. N. R., *et al.* (2005). *J. Biol. Chem.* **280**, 22793-22799.
26174	Sulfo-LC-SDA **M.W. 440.40** **Spacer Arm 12.5Å**		
21650	Sulfo-LC-SPDP **M.W. 527.57** **Spacer Arm 15.7Å**		• Zhao, D., *et al.* (2011). *Clin. Cancer Res.* **17**, 771-782. • Zhang, J., *et al.* (2008). *Mol. Cell. Proteomics* **7**, 1378-1388. • Backer, M. V., *et al.* (2005). *Mol. Cancer Ther.* **4**, 1423-1429.
22312	Sulfo-MBS **M.W. 416.30** **Spacer Arm 7.3Å**		• Adessi, C., *et al.* (2000). *Nucleic Acids Res.* **28**, e87. • Özvegy-Laczka, C., *et al.* (2008). *J. Biol. Chem.* **283**, 26059-26070. • Faye, A., *et al.* (2007). *J. Biol. Chem.* **282**, 26908-26916.
26777	Sulfo-NHS-Acetate **M.W. 259.17**		
88906	Sulfo-NHS-Phosphine **M.W. 563.45**		
22589	Sulfo-SANPAH **M.W. 492.40** **Spacer Arm 18.2Å**		• Oakes, P. W., *et al.* (2012). *J. Cell Biol.* **196**, 363-374. • Elliott, C. G., *et al.* (2012). *J. Cell Sci.* **125**, 121-132. • Mierke, C. T., *et al.* (2011). *J. Biol. Chem.* **286**, 34858-34871. • Liu, Z. et al. (2011). *J. Biol. Chem.* **286**, 30795-30805. • Betz, T., et al. (2011). *Proc. Natl. Acad. Sci. USA* **108**, 13420-13425.

Chemical Structures

Product #	Product Name	Structure	Reference
33033 33034	**Sulfo-SBED** **M.W. 879.98** **Spacer Arms** **Sulfo-NHS ester 13.7Å** **Phenyl azide 9.1Å** **Biotin 19.1Å**		• Emara, M., *et al.* (2012). *J. Biol. Chem.* **287**, 5756-5763. • Gabriele Loers, G., *et al.* (2012). *J. Neurosci.* **32**, 3917-3930. • Benjamin Moore, B., *et al.* (2011). *J. Biol. Chem.* **286**, 9246-9256.
26173	**Sulfo-SDA** **M.W. 327.25** **Spacer Arm 3.9**		
22327	**Sulfo-SIAB** **M.W. 514.19** **Spacer Arm 10.6Å**		• Härmä, H., *et al.* (2000). *Clin. Chem.* **46**, 1755-1761. • Adessi, C., *et al.* (2000). *Nucleic Acids Res.* **28**, e87. • Allersom, C., R., *et al.* (2003). *RNA* **9**, 364. • Crawford, J. B. and Patton, J. G. (2006). *Mol. Cell. Biol.* **26**, 8791-8802.
22322 22122 22622	**Sulfo-SMCC** **M.W. 436.37** **Spacer Arm 8.3Å**		• Harvey, S., *et al.* (1998). *Clin. Chem.* **44**, 509-516. • Adessi, C., *et al.* (2000). *Nucleic Acids Res.* **28**, e87. • Betting, D. J., *et al.* (2008). *J. Immunol.* **181**, 4131-4140. • Mamedova, A. A., *et al.* (2004). *J. Biol. Chem.* **279**, 23830-23836. • Percy, A. J., *et al.* (1996). *Clin. Chem.* **42**, 576-585.
22317	**Sulfo-SMPB** **M.W. 458.38** **Spacer Arm 11.6Å**		• Zhu, J-X., *et al.* (2005). *J. Biol. Chem.* **280**, 32468-32479. • Mamedova, A. A., *et al.* (2004). *J. Biol. Chem.* **279**, 23830-23836. • Goldoni, S., *et al.* (2004). *J. Biol. Chem.* **279,** 6606-6612. • Niemeyer, C. M., *et al.* (2003). *Nucleic Acids Res.* **31**, e90. • Pu, W. T., *et al.* (1999). *Mol. Cell. Biol.* **19**, 4113-4120.
77720	**TCEP Solution, Neutral pH** **M.W. 250.2**		• Irsch, T. and Krauth-Siegel, R.L. (2004) *J. Biol. Chem.* **279**, 22209-22217. • Schmidt, H. and Krauth-Siegel, R.L. (2003) *J. Biol. Chem.* **278**, 46329-46336.

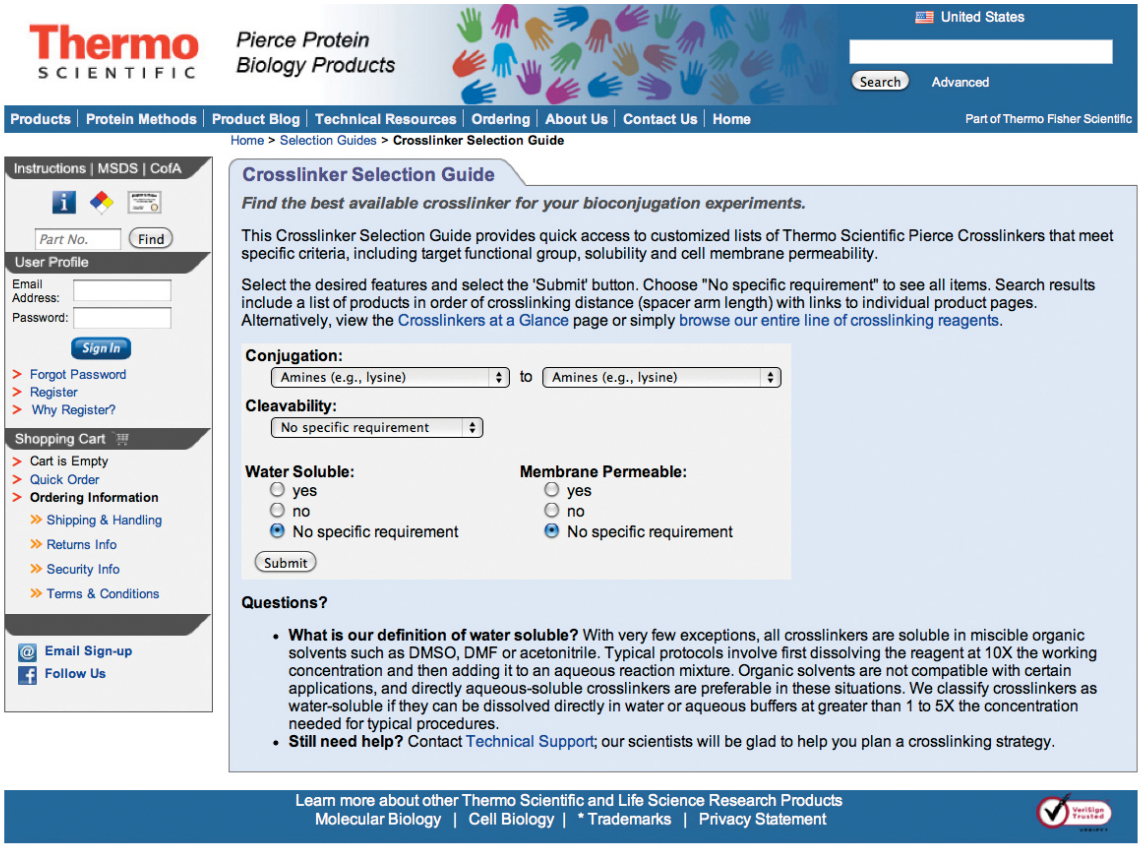
Chemical Structures

Product #	Product Name	Structure	Reference
22299	**TFCS** **M.W. 324.25** **Spacer Arm 7.7Å**		
33043	**TMEA** **M.W. 386.36** **Spacer Arm 10.3Å**		• Auclair, J. R., *et al.* (2010). *Proc. Natl. Acad. Sci. USA* **107**, 21394-21399. • Gosink, K. K., *et al.* (2011). *J. Bacteriol.* **193**, 6452-6460. • Studdert, C. A. and Parkinson, J. S. (2004). *Proc. Natl. Acad. Sci. USA* **101**, 2117-2122.
22361	**TMM(PEG)$_{12}$** **M.W. 2,630.8** **Spacer Arm 77.6Å**		• Hermanson, G.T. (2008). *Bioconjugate Techniques*, Academic Press • Harris, J. M. and Zalipsky, S. Eds (1997). ACS Symposium Series, 680. • Harris, J. M. and Kozlowski, A. (2001). *J. Control Release* **72**, 217-224. • Veronese, F. and Harris, J.M. Eds. (2002). *Advanced Drug Delivery Review* **54(4)**, 453-609.
22421 **22424**	**TMS(PEG)$_{12}$** **M.W. 2,420.8** **Spacer Arm 77.5Å**		• Hermanson, G.T. (2008). *Bioconjugate Techniques*, Academic Press. • Harris, J. M. and Zalipsky, S. Eds (1997). ACS Symposium Series, 680. • Harris, J. M. and Kozlowski, A. (2001). C. *J. Control Release* **72**, 217-224. • Veronese, F. and Harris, J.M. Eds. (2002). *Advanced Drug Delivery Review* **54(4)**, 453-609.
26101	**Traut's Reagent** **M.W. 137.63** **Spacer Arm 8.1Å**		• Traut, R.R., *et al.* (1973). *Biochem* **12(17)**, 3266-73. • Jue, R., *et al.* (1978). *Biochem* **17(25)**, 5399-5405. • D'Oro, U., *et al.* (2002). *J Immunol* **169**, 6269-78. • Newton, D.L., *et al.* (2001). *Blood* **97**, 528-35. • Stanisic, D.I., *et al.* (2003). *Infec Immunity* **71(10)**, 5700-13.
33063	**TSAT** **M.W. 482.36** **Spacer Arm 4.2Å**		• Bofill-Cardona, E., *et al.* (2000). *J. Biol. Chem.* **275**, 32672-32680.
29700	**Urea** **M.W. 60.06**		• Hames, B.D. and Rickwood, D., eds. (1984) *Gel Electrophoresis of Proteins: A Practical Approach*. Washington D.C. : IRL Press. • Lacks, S.A. , *et al.* (1979) *Anal. Biochem.* **100**, 357-363.

appendix 2

Online interactive crosslinker selection guide

We have developed an interactive crosslinker selection guide to aid in deciding which crosslinker is the best for your application. Go to www.thermoscientific.com/pierce, choose "selection guides" from the Technical Resources drop-down menu and then choose the crosslinker selection guide. The interactive selection guide will guide you through the process of choosing the appropriate crosslinker for your application.



Glossary of crosslinking terms

Acylation: Reaction that introduces an acyl group (-COR) into a compound.

Aryl azide: Compound containing a photoreactive functional group (e.g., phenyl azide) that reacts nonspecifically with target molecules.

Carbodiimide: Reagent that catalyzes the formation of an amide linkage between a carboxyl (–COOH) group and a primary amine ($–NH_2$) or a hydrazide ($–NHNH_2$). These reagents do not result in the formation of a cross-bridge and have been termed zerolength crosslinkers.

Crosslinker: A reagent that will react with functional groups on two or more molecules to form a covalent linkage between the molecules.

Conjugation reagent: A crosslinker or other reagent for covalently linking two molecules.

Diazirine crosslinker: The succinimidyl-ester diazirine (SDA) crosslinkers combine amine-reactive chemistry with an efficient diazirine-based photochemistry for photo-crosslinking to nearly any other functional group. The photoactivation of diazirine with long wave UV light (330-370 nm) creates carbene intermediates. These intermediates can form covalent bonds via addition reactions with any amino acid side chain or peptide backbone at distances corresponding to the spacer arm lengths.

Disulfide bonds: Oxidized form of sulfhydryls (- S–S -); formed in proteins through –SH groups from two cysteine molecules. These bonds often link polypeptide chains together within the protein and contribute to a protein's tertiary structure.

α-Haloacyl: Functional group (e.g., iodoacetyl) that targets nucleophiles, especially thiols. α-Haloacyl compounds have a halogen atom such as iodine, chlorine or bromine attached to an acyl group on the molecule. These alkylating reagents degrade when exposed to direct light or reducing agents, resulting in the loss of the halogen and the appearance of a characteristic color.

Hapten: A molecule recognized by antibodies but unable to elicit an immune response unless attached to a carrier protein. Haptens are usually, but not always, small (< 5 kDa) molecules.

Homobifunctional crosslinker: Reagent with two identical reactive groups used to link two molecules or moieties.

Heterobifunctional crosslinker: Reagent with two different reactive groups used to link two molecules or moieties.

Hydrophilic: Substances that readily dissolve in water.

Hydrophobic: Substances with limited solubility in water.

***N*-Hydroxysuccinimidyl (NHS) ester:** Acylating reagents commonly used for crosslinking or modifying proteins. They are specific for primary ($–NH_2$) amines between pH 7-9, but are generally the most effective at neutral pH. These esters are subject to hydrolysis, with half-lives approximating one to two hours at room temperature at neutral pH.

Imidate crosslinker: Primary amine-reactive functional group that forms an amidine bond. The ε-amine in lysine and N-terminal amines are the targets in proteins. Imidates react with amines in alkaline pH conditions (pH range 7.5-10) and hydrolyze quickly, with half-lives typically around 10-15 minutes at room temperature and pH 7-9. At pH > 11, the amidine bond is unstable, and crosslinking can be reversed. The amidine bond is protonated at physiological pH; therefore, it carries a positive charge.

Imidoester: Amine-reactive functional group of an imidate crosslinker.

Immunogen: A substance capable of eliciting an immune response.

Integral membrane protein: Protein that extends through the cell membrane and is stabilized by hydrophobic interactions within the lipid bilayer of the membrane.

Ligand: A molecule that binds specifically to another molecule. For example, a protein that binds to a receptor.

Moiety: An indefinite part of a sample or molecule.

Monomer: Consisting of a single unit.

NHS: Abbreviation for ***N***-hydroxysuccinimide.

Nitrene: Triple-bonded nitrogen-to-nitrogen reactive group formed after exposure of an azido group to UV light. Its reactivity is nonspecific and short-lived.

Nonselective crosslinking: Crosslinking using a reactive group, such as nitrenes or aryl azides, which react so quickly and broadly that specific groups are not easily and efficiently targeted. Yields are generally low with many different crosslinked products formed.

Nonspecific crosslinking: Another term for nonselective crosslinking.

Oligomer: A molecule composed of several monomers.

Photoreactive: A functional group that becomes reactive upon excitation with light at a particular range of wavelengths.

Polymer: A molecule composed of many repeating monomers.

Pyridyl disulfide: Aromatic moiety with a disulfide attached to one of the carbons adjacent to the nitrogen in a pyridine ring. Pyridine 2-thione is released when this reagent reacts with a sulfhydryl (–SH)-containing compound.

Spacer arm: The part of a crosslinker that is incorporated between two crosslinked molecules and serves as a bridge between the molecules.

Substrate: A substance upon which an enzyme acts.

Sulfhydryl: –SH groups present on cysteine residues in proteins.

Thiols: Also known as mercaptens, thiolanes, sulfhydryls or –SH groups, these are good nucleophiles that may be targeted for crosslinking.

Ultraviolet: Electromagnetic radiation of wavelengths between 10-390nm.

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