Click Chemistry Tools
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Bis-sulfone Reagents

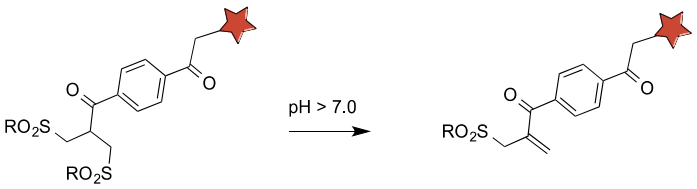
An intact IgG molecule has four accessible inter–chain disulfide bonds that can be reduced to form eight free cysteine thiols, which can serve as sites for conjugation. The reaction between reduced IgG and maleimide reagents yields conjugates composed of a mixture of species with between 0 and 8 payload moieties per antibody.

Maleimide–based conjugates have been shown to undergo retro–addition reactions in the presence of competing thiols. In serum, this can result in de–conjugation by exchange reactions with free thiol groups within circulating albumin. Maleimide, as well as other monoalkylation reagents also leave the cysteines from the original disulfides unbridged, introducing a potential for instability to the antibody.

A promising solution to these problems is the utilization of bis–reactive bridging reagents that are able to restore a covalent linkage between the two cysteines. This approach uses bis–sulfone reagents that are selective for the cysteine sulfur atoms from a native disulfide that has been reduced **(Figure 2)**.

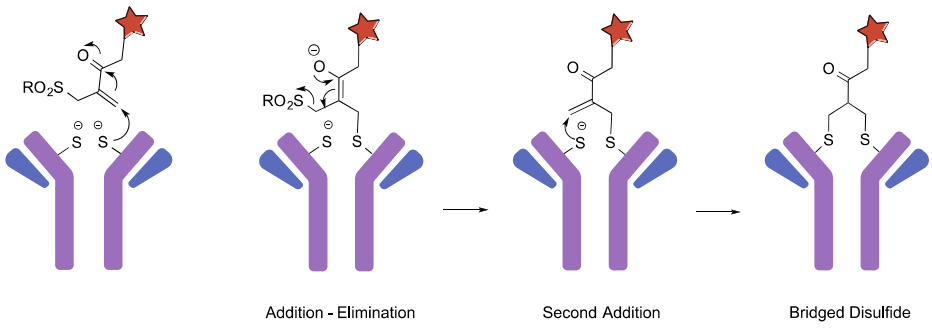
Mechanistically, the first step is elimination of sulfinic acid to form a conjugated double bond to initiate a sequence of addition-elimination reactions (**Figure 1)**.

Figure 1.



After initial Michael addition of the first thiol, a second elimination of sulfinic acid generates another conjugated double bond for the addition of second thiol. This leads to the formation of a three-carbon bridge between two cysteine residues of a reduced native disulfide bond such as the interchain disulfide bonds of a mAb. The reaction results in covalent re-bridging of the disulfide bond via a three-carbon bridge leaving the protein structurally intact.

Figure 2.



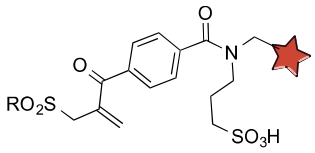
ThioLinker™ Reagents

The bis–sulfone reagents have been known for almost 25 years, however their application was mostly limited to conjugation of hydrophilic PEGs to proteins. The limited application of these reagents is associated with their rather poor re–bridging and conjugation efficiency, which can be attributed to the high hydrophobicity of bis–sulfone reagents.

Often, functionalization of hydrophobic toxins with a bis-sulfone moiety makes the resulting conjugates practically insoluble in aqueous media. To overcome the solubility issue, large amount of organic co-solvents should be used to achieve desired degree of labeling and re-bridging.

We have developed unique, water-soluble bis-alkylating reagents that overcome the drawbacks associated with traditional bis–sulfone reagents **(Figure 3)**. Incorporation of sulfopropyl groups greatly improves not only solubility but also labeling and re-bridging efficiency. In addition, upon functionalization of a payload with water-soluble bis-alkylating reagents, the aqueous solubility of resulting conjugate is greatly improved compared to unmodified molecule.

Figure 3.

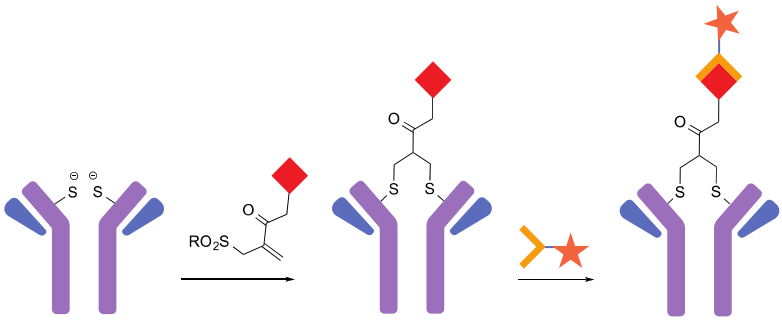


Several amine, and carboxyl reactive building blocks and clickable reagents are available from Click Chemistry Tools as off-shelf products. Upon request our team of chemists can prepare custom ThioLinker™ reagents according to your specification.

Two-step Approach

This approach uses novel bis–sulfone reagents to introduce a small bioorthogonal group. The payload, which is equipped with a complementary functional group reactive toward the linker, is then reacted with the protein–linker conjugate to yield the desired ADC. In this approach, the first step takes advantage of the selectivity of the bridging reagents, which is used to introduce a new bioorthogonal reactive group that then provides superior reaction kinetics for a second, more complex conjugation reaction. A two–step strategy that employs bioorthogonal ligation chemistry is outlined in **Figure 4**.

Figure 4.



A distinct advantage of a two-step approach is that it is compatible with payloads of any complexity and size as attachment of the payload is done independently from antibody modification. Another advantage of this approach is that it allows for the utilization of different payloads without altering the antibody modification step. In addition, using our meticulously designed, off-shelf ThioLinker™ 'clickable' reagents, very little or no optimization is required to prepare homogeneous ADCs.

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