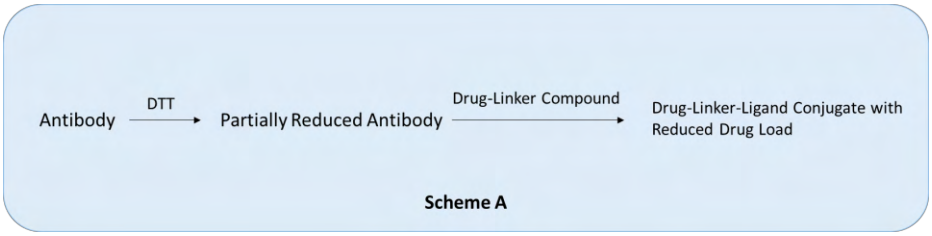
General Antibody Drug Conjugate Protocol



Scheme A illustrates methodology useful for making Drug-Linker-Ligand conjugates having about 2 to about 4 drugs per antibody.

General Procedure: Preparation of Conjugates Having about 2 to about 4 Drugs Per Antibody.

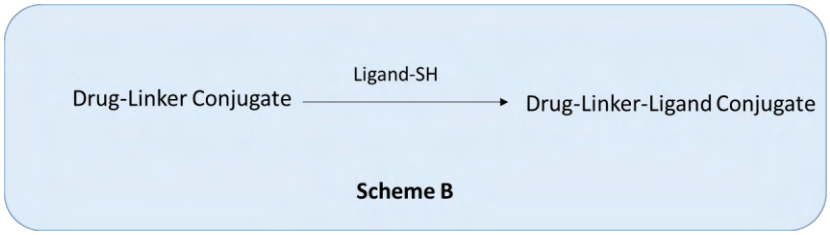
Partial Reduction of the Antibody

In general, to prepare conjugates having 2 drugs per antibody, the relevant antibody is reduced using a reducing agent such as dithiothreitol (DTT) or tricarbonyl ethylphosphine(TCEP)(about 1.8 equivalents)in PBS with 1 mM DTPA, adjusted to pH8 with 50 mM borate. The solution is incubated at 37° C. for 1 hour, purified using a 50 ml G25 desalting column equilibrated in PBS/1 mM DTPA at 4°C. The thiol concentration can be determined according to General Procedure M, the protein concentration can be determined by dividing the A280 value by 1.58 extinction coefficient (mg/ml), and the ratio of thiol to antibody can be determined according to General Procedure N.

Conjugates having 4 drugs per antibody can be made using the same methodology, using about 4.2 equivalents of a suitable reducing agent to partially reduce the antibody.

Conjugation of Drug-Linker to Partially Reduced Antibody

The partially reduced antibody samples can be conjugated to a corresponding Drug-Linker compound using about 2.4 and about 4.6 molar equivalents of Drug-Linker compound per antibody to prepare the 2 and 4 drugs per antibody conjugates, respectively. The conjugation reactions are incubated on ice for 1 hour, quenched with about 20-fold excess of cysteine to drug, and purified by elution over a G25 desalting column at about 4°C. The resulting Drug-Linker-Ligand conjugates are concentrated to about 3 mg/ml, sterile filtered, aliquoted and stored frozen.



Scheme B depicts the construction of a Drug-Linker-Ligand Conjugate by reacting the Sulfhydryl group of a Ligand with a thiol-acceptor group on the Linker group of a Drug-Linker Compound.

Illustrative methods for attaching a Ligand antibody to a Drug-Linker Compound are outlined below in General Procedures L-M.

General Procedure L: Attachment of an Antibody Ligand to a Drug-Linker Compound

All reaction steps are typically carried out at 4°C. Where the Ligand is a monoclonal antibody having one or more disulfide bonds, solutions of the monoclonal antibody (5-20 mg/mL) in phosphate buffered saline, pH 7.2, are reduced with dithiothreitol (10 mM final) at 37° C. for 30 minutes (See General Procedure M) and separation of low molecular weight agents is achieved by size exclusion chromatography on Sephadex G25 columns in PBS containing 1 mM diethylenetriaminepentaacetic acid.

The sulfhydryl content in the Ligand can be determined using 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) as described in General Procedure M *(see Riddles, P. W., Blakeley, R. L., and Zerner, B. (1979) Anal. Biochem. 94, 75-81)*.

To a PBS solution of Ligand reduced according to General Procedure L, a Drug-Linker Compound in MeCN is added so that the solution is 20% MeCN/PBS (vol/vol). The amount of Drug-Linker Compound is approximately 10% more than the total number of sulfhydryl groups on a Ligand. After 60 min at 4°C., cysteine is added (20-fold excess over concentration of the Drug-Linker Compound), the Solution is concentrated by ultrafiltration, and any low molecular weight agents are removed by gel filtration

The number of Drug-Linker Compounds per antibody is determined by UV/vis spectroscopy using formulas derived from the relative extinction coefficients of the Ligands and Drug-Linker Compounds as described in General Procedure O. The amount of quenched Drug-Linker Compound is then determined as described in General Procedure P using reverse-phase HPLC. The aggregation state of the Ligand Antibodies of the Drug-Linker-Ligand Conjugates can be determined using size-exclusion HPLC as described in General Procedure P. The Drug-Linker-Ligand Conjugates can be used without further purification.

General Procedure M: Reduction of the interchain disulfide bonds of an Antibody.

To a solution of 24 mg of an antibody (2.4 mL of 10 mg/mL solution) in suitable buffer is added 300 uL of Borate buffer (500 mM sodium borate/500mM sodium chloride, pH 8.0) followed by 300 uL of Dithiothreitol (DTT, 100 mM solution in HO). The reaction mixture is stirred using a vortex instrument and incubated at 37°C. for 30 min. Three PD10 columns are equilibrated with PBS containing 1 mM DTPA (in PBS) and the reduced antibody is eluted through the three PD10 columns and collected in 4.2mL PBS/DTPA solution (1.4 mL per column). The reduced antibody is then stored on ice. The number of thiols per antibody and the antibody concentration are determined according to General Procedure N.

Reference:

Senter, Peter D., Svetlana O. Doronina, and Brian E. Toki. Drug Conjugates and Their Use for Treating Cancer, an Autoimmune Disease or an Infectious Disease. Seattle Genetics Inc, assignee. Patent US7659241B2. 31 July 2002. Print.