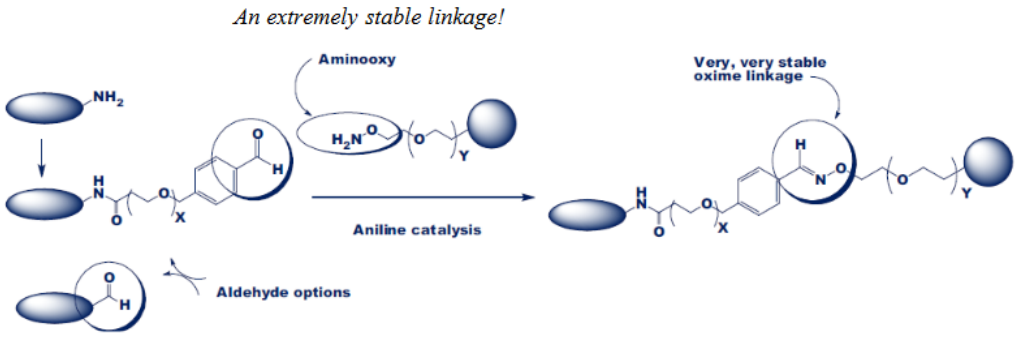
PEG Ald Reagents

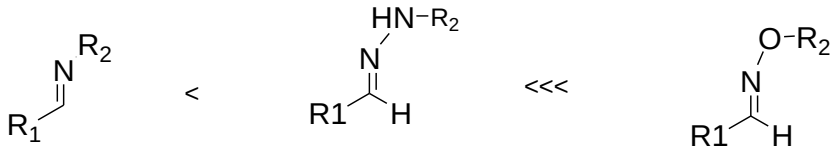
Introduction

PEG Ald reagents can be used in bioconjugation through the reaction of the aldehyde group with aminooxy to form aldoxime. It is much more stable than hydrazone and imine. In most of cases, it can be used directly without reduction while hydrazine and imines bond normally need be reduced to form stable C-N bond.

Alkoxyamines react with carbonyls most efficiently in amine-free, neutral conditions (pH 6.5-7.5). Carbonyls may exist at the reducing end of polysaccharides. To create additional carbonyls, oxidize sugar groups using either a specific oxidase, such as galactose oxidase, or 1-10mM sodium meta-periodate or use Ald-PEG-NHS ester to introduce a aldehyde group. Oxidation with periodate is most efficient in acidic conditions (e.g., 0.1M sodium acetate, pH 5.5), although neutral buffers such as phosphate- buffered saline can also be used. If oxidation is performed in acidic conditions, buffer exchange by dialysis or gel filtration into neutral buffer might be necessary to obtain the optimal alkoxyamine reaction. Sometimes, aniline can be used to accelerate the coupling rate of hydrazide and alkoxyamine moieties with reactive aldehydes / ketones (carbonyls).



b. stability



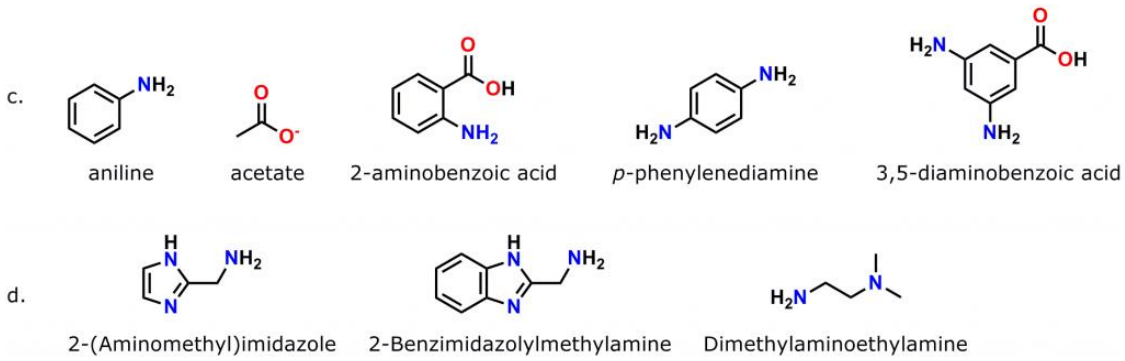


Figure 1: Oxime bond formation is a specific, bioorthogonal reaction that can be greatly accelerated by the use of suitable catalysts.

a. Scheme for the formation of aldoximes, when an aminooxy compound reacts with an aldehyde.
b. stability comparison of imine, hydrazone, and oxime.
c. Common catalysts used to accelerate oxime bond formation.
d. Recently discovered low-toxicity catalysts for oxime bond formation.

Example Protocol for labeling glycoproteins with an alkoxyamine-biotin reagent

Note: The optimal alkoxyamine-biotin concentration and reaction conditions depend on the specific protein and downstream application. For best results, empirically optimize the molar ratio of reagent and glycoprotein.

A. Materials required

- Alkoxyamine-biotin Solution: 50mM alkoxyamine-biotin reagent in DMSO. Prepare a volume sufficient to achieve the desired final concentration in step B.3.
  Note: Alkoxyamine biotin reagents are hygroscopic solids that are difficult to weigh and dispense. To facilitate handling, make a 250mM stock solution in DMSO. Store the stock solution at -20°C for up to 1 month; warm the vial to room temperature before opening to prevent moisture condensation.
- Coupling Buffer: 0.1M sodium phosphate, 0.15M sodium chloride; pH 7.2 (PBS) or other neutral or slightly alkaline, non-amine buffer
- Glycoprotein at 2mg/mL
- Dialysis cassette or desalting column

B. Procedure

1. 100 µg of Glycoprotein is mixed with 5 µl of 1M $NaHCO_3$ with 100 µl of the PBS-based solution.
   20 nmol of Ald-PEG-NHS ester is added to the mixture. The reaction mixture is kept at room temperature for 60 minutes. Desalting procedure is followed by using spin desalting column. The recovery protein amount after desalting was calculated as ~75 µg.
2. Add 15X alkoxyamine-biotin solution to activated protein above. Mix the reaction for 2 hours at room temperature.
3. Separate the biotinylated protein from non-reacted material by dialysis or desalting. Store the biotinylated protein using the same conditions as for the non-biotinylated sample.