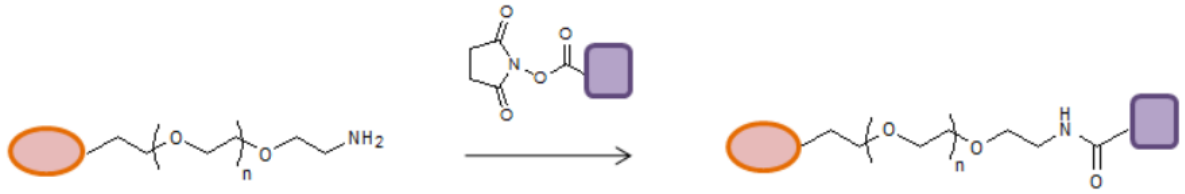
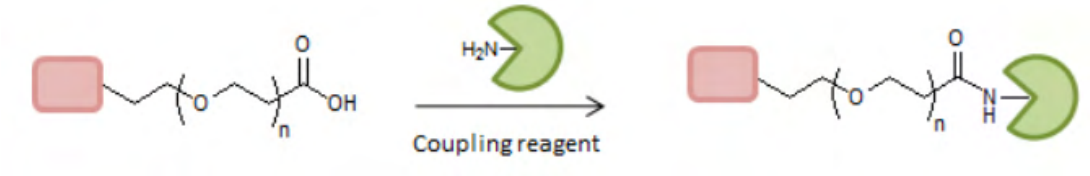
Instructions for Amino PEG Reagents



No coupling reagent is required. The PEG amine can perform efficient PEGylation with NHS ester at pH 7-9.

1) Slowly dissolve amine bearing small molecules in organic solvents such as DMF, CH_2Cl_2, DMSO, THF, or other solvents as needed.
2) Under continuous stirring, NHS-containing compound was added to the above reaction mixture 1:1 or 2:1 equivalent by mmol depending on the reaction kinetics.
3) The reaction mixture was stirred for 3-24 hours depending on the substrate properties, monitored either by LC-MS or TLC plate.
4) The final product can be isolated by general organic synthesis workup or by column purification.



Ratio 1:1 equivalent

Coupling reagents could be EDC, DCC, HATU. EDC crosslinking is most efficient in acidic (pH 4.5) conditions.

1) Equilibrate EDC and carboxylic acid to room temperature before opening bottles.
2) Prepare carboxylic acid stock solutions by dissolving 100mg of each reagent (~100µL) in the desired amount of dry water-miscible solvent (e.g., DMF or DMSO).
3) Cap, store and handle stock solutions as directed in the Important Product Information Section.
4) Add appropriate amounts of EDC and amine-containing molecule to the appropriate amount of carboxylated surface in Activation Buffer and react for 15 minutes at room temperature.
5) Add DTT to quench the EDC. Note: For surfaces that can be easily washed, the quenching step can be skipped, and the surface washed with Coupling Buffer to remove any remaining EDC and NHS.
6) Add the carboxylic acid mixture prepared in Conjugation Buffer to the activated surface and react for 2 hours at room temperature.
7) To quench the reaction, add hydroxylamine or another amine-containing buffer. Hydroxylamine hydrolyzes non-reacted NHS on the solid surface and results in hydroxamate formation. Other quenching methods involve adding Tris, lysine, glycine or ethanolamine; however, these primary amine-containing compounds modify carboxyls.
   (Note: The newly introduced carboxy groups can be further modified by repeating Steps 4 and 5)
8) Add the desired amine-containing substrate, prepared in Coupling Buffer, to the activated surface and react for 2 hours at room temperature.
9) Quench the reaction as described in Step 7.