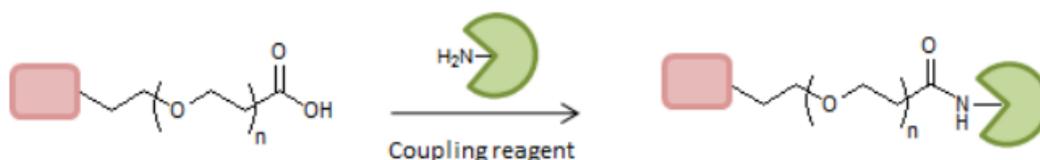


No coupling reagent is required. The PEG amine can perform efficient PEGylation with NHS ester at pH 7-9.

- 1) Slowly dissolve amine bearing small molecules in organic solvents such as DMF, CH<sub>2</sub>Cl<sub>2</sub>, 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.