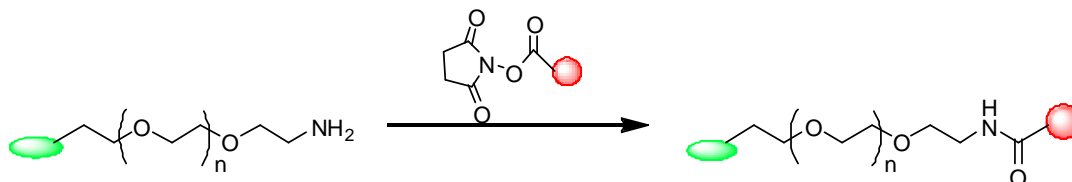


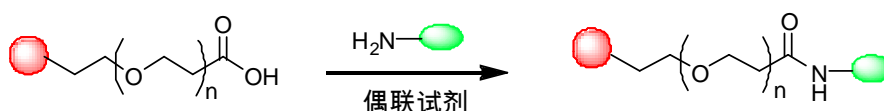
Amino PEG

Protocol



Does not require coupling agents. When PH value is 7-9, PEG amine can react with NHS ester effectively.

- (1) Slowly dissolve amines containing small molecules in organic solvents, such as DMF, CH₂Cl₂, DMSO, THF, or other solvents.
- (2) According to the reaction kinetics, the NHS-containing compound was added to the reaction mixture in 1:1 or 2:1 equivalent by mmol under continuous agitation.
- (3) The reaction mixture was stirred for 3-24 h according to the nature of the substrate and monitored by LC-MS or TLC plate.
- (4) The final product can be separated by conventional organic synthesis or column purification.



Ratio 1: 1 equivalent

Coupling reagents can be EDC, DCC, HATU. EDC crosslinking is most effective under acidic (pH 4.5) conditions.

- (1) Keep EDC and carboxylic acid to room temperature before opening the bottle.
- (2) A reserve solution of carboxylic acid is prepared by dissolving 100mg of each reagent (~ 100 μ L) in a required amount of dry solvent (such as DMF or DMSO) miscible with water.
- (3) Appropriate amounts of EDC and amine-containing molecules were added to the appropriate amount of carboxylated surface in the activation buffer, and reacted at room temperature for 15 minutes.
- (4) Add DTT to quench EDC. Note: For surfaces that are easy to clean, the quenching step can be skipped and the surface washed with coupling buffer to remove any remaining EDC and NHS.
- (5) The carboxylic acid mixture prepared in the conjugate buffer was added to the activated surface and reacted at room temperature for 2 hours.
- (6) Add hydroxylamine or another amine-containing buffer to quench the reaction. Hydroxylamine hydrolyzes unreacted NHS on a solid surface and forms a hydroxylate salt. Other methods of quenching include the addition of melamine, lysine, glycine or

ethanolamine; however, these primary amine-containing compounds can modify carboxyl groups. (Note: The newly introduced carboxyl group can be further modified by repeating steps 4 and 5)

(7) The desired amine-containing substrate prepared in the coupling buffer was added to the activated surface and reacted at room temperature for 2 hours.

(8) Quench the reaction as described in step 7.