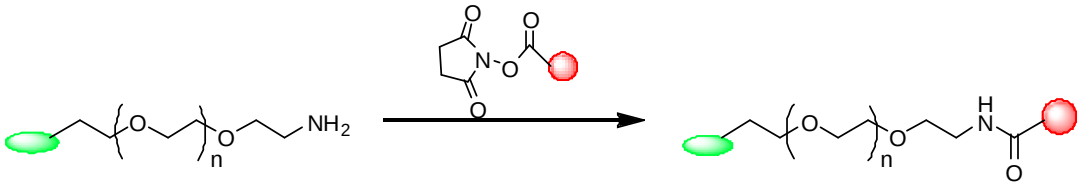
Amino PEG

Protocol



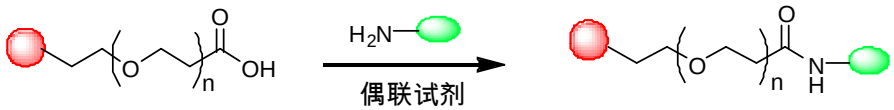
Does not require coupling agents. When PH value is 7-9, PEG amine can react with NHS ester effectively.

(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.