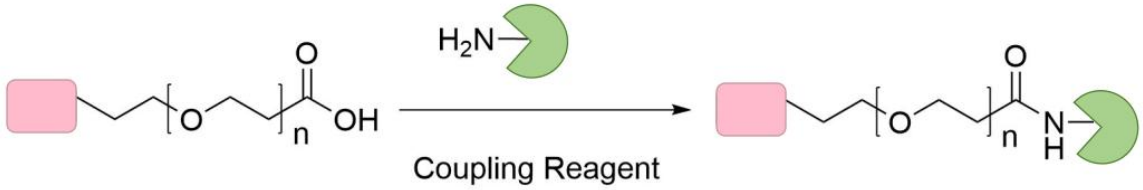
PEG Acid Reagents

Introduction

PEG acid is a type of PEG compound that features a carboxylic acid group at one end and can have a hydroxyl, azido, amino, maleimide, or triple bond at the other end. These reagents come with specific molecular weights and spacer lengths, making them suitable for modifying proteins or surfaces with amine groups, such as quantum dots, self-assembled monolayers, and magnetic particles. Using PEG spacers to functionalize solid surfaces greatly minimizes nonspecific protein binding.



Condition: 1 a) EDC, NHS, DMF or DCM, b) R-NH2

Condition 2. a) 0.5mM MES buffer, pH4.5, EDC, NHS; b) R-NH2

Product Information

Note: Upon receipt store desiccated at -20°C.

- Most PEG Acid reagents are low-melting solids that are challenging to weigh and dispense. To make handling easier, prepare a stock solution by dissolving the reagent in dimethylsulfoxide (DMSO) or dimethylformamide (DMF).
- Store any unused stock solution at -20°C. Before opening the reagent vial, allow it to reach room temperature to prevent moisture condensation. To reduce air exposure, keep the stock solution under an inert gas like argon or nitrogen. Seal the stock solution with a septum and use a syringe to withdraw the solution.
- When the PEG Acid is used for surface binding and further protein loading, the reagent-to-surface ratio in the reaction affect the number of carboxylic acid residues available for further modification. Optimize these ratios to obtain the modification level needed for the specific application.
- Use buffers that do not contain amines, with a pH range of 7-9, such as PBS (20mM sodium phosphate, 150mM NaCl; pH 7.4), 100mM carbonate/bicarbonate, or 50mM borate. Avoid buffers containing primary amines, like Tris or glycine, as they compete with acylation.

Additional Materials Required

Water-miscible organic solvent (molecular sieve-treated) such as DMSO or DMF; Small-volume, non-coring syringes for dispensing the reagent stock solution while minimizing exposure to air; PBS Buffer Phosphate-buffered saline, PBS (20mM sodium phosphate, 0.15MNaCl; pH 7.2) or other non-amine, lone- pair sulfur-free buffers; MES Buffer: MES-buffered saline (0.1M MES, 0.5M NaCl; pH 6.0 or 0.1M MES, 0.9% NaCl; pH 4.7); EDC.HCl; NHS; Hydroxylamine; HCl

Address: 5820 Oberlin Drive, Suite 104,San Diego, California 92121, USA
Phone: 1-858-677-9432; **fax**: 1-858-228-1081
Web: https://axispharm.com/
Email: sales@axispharm.com

Procedure (organic solvent method)

PEG acid (1 mmol) was dissolved in 1.5 mL of dry DCM. EDC·HCl (2.0 mmol) was dissolved by 0.5 mL of dry DCM, and NHS (2.0 mmol) was dissolved by 10 µL of DMSO; both were added sequentially to the solution. The mixture was stirred at room temperature for 30 minutes. Subsequently, NH2-R' (1.5 mmol) was added to the solution, followed by the addition of DIPEA (1.5 mmol). The mixture was then stirred at room temperature for 1 hour. The reaction mixture can be purified using flash R18 chromatography to obtain the pure compound.

Procedure (aqueous method)

1. Allow the PEG Acid reagents to reach room temperature before opening the bottles.

2. Create stock solutions by dissolving 100 mg of each reagent in the desired amount of DMF or DMSO. Cap, store, and handle the stock solutions according to the guidelines in the Important Product Information Section.

3. Prepare the required amount of surface or protein in PBS buffer.

4. Activate the carboxylic acid groups on the PEG linker by adding appropriate amounts of EDC and NHS to the modified surface in a small amount of Buffer B, and let it react for 15 minutes at room temperature. For optimal results, conduct this reaction at pH 5-6.

Note: The activation reaction with EDC and NHS is most efficient at pH 4.5-7.2; however, the reaction of NHS-activated molecules with primary amines is most efficient at pH 7-8.

5. Add the desired amine-containing substrate, prepared in PBS buffer, to the activated surface and let it react for 2 hours at room temperature. For best results, adjust the pH of the reaction solution to 7.2-7.5 with PBS buffer immediately before adding the amine-containing substrate.

6. To quench the conjugation reaction, add hydroxylamine or another amine-containing buffer. Hydroxylamine hydrolyzes non-reacted NHS. Other quenching agents include Tris, lysine, glycine, or ethanolamine; however, these primary amine-containing compounds will modify carboxylic acids.

References

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