

Polyester bis-MPA dendrons and dendrimers are available from Polymer Factory with NHBOC protected amine functionality, with the dendrons having a unique ability to act as bifunctional linkers in biochemistry and materials science. These are available with a range of orthogonal reactive groups and can act as signal amplifiers, bearing a precise number of reactive groups at the periphery.

The NHBOC protecting group can be quantitatively deprotected to reveal ammonium groups for further reaction (SOP available). The primary amine groups at the periphery or dendron focal point are ideal for common coupling methods used in bio-conjugation, such as amine-NHS reaction, with the dendron also containing additional reactive site(s) for further modification *via*, for example, click chemistry.

Functionalization of amine with model NHS-ester

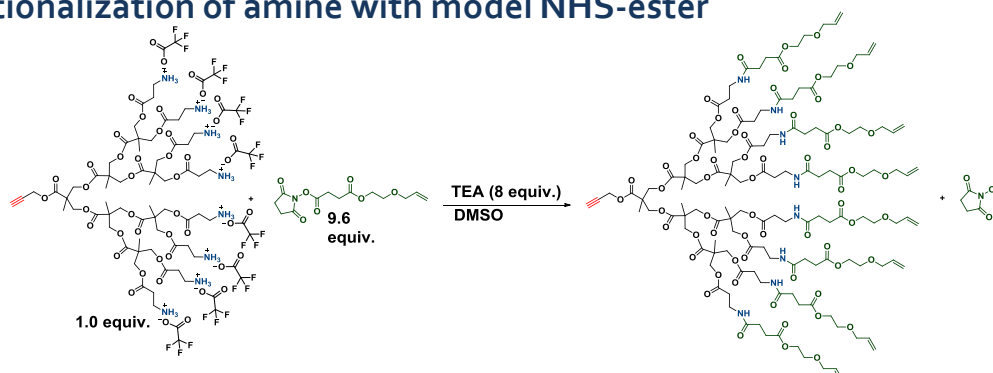


Figure 1: Reaction scheme of amine functional dendron modification with NHS derivatives

Protocol (with DMSO as solvent)

- Dissolve the dendron (1 equiv.) and TEA (8 equiv., 1 per amine) in DMSO.
- Add the NHS-derivatives (≥ 1.2 equiv. per amine) to the reaction solution and stir at room temperature.
- Follow the reaction by MALDI-TOF MS. The increase in molecular weight can be observed where each peak corresponds to the attachment of NHS derivatives to the dendron. The MALDI-TOF MS spectrum will show the monodisperse peak at the final molecular weight, figure 3c.

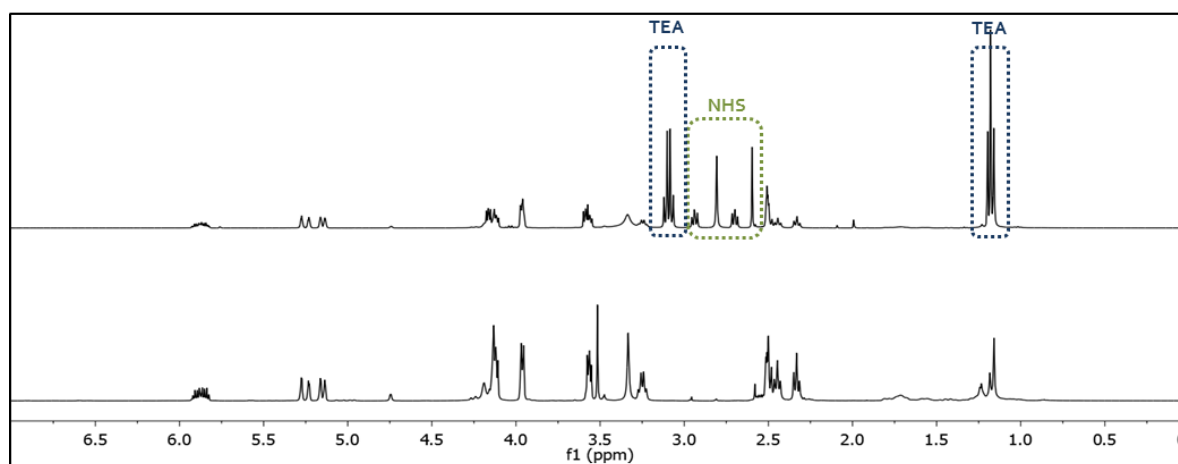


Figure 2: $^1\text{H-NMR}$ spectra in $\text{DMSO-}d_6$ of a) the reaction mixture and b) product after purification

- After completion, quench the excess of NHS derivatives with deionized water. Quenching can be followed by ¹H-NMR. The excess of NHS derivatives and free NHS correspond to the peaks at 2.59 and 2.80 ppm, and will be absent (Figure 2). Quenching may take several hours.
- Add EtOAc and wash the reaction with NaHSO₄ 10% aq., NaHCO₃ 10% aq. and brine. Dry the solution with MgSO₄.

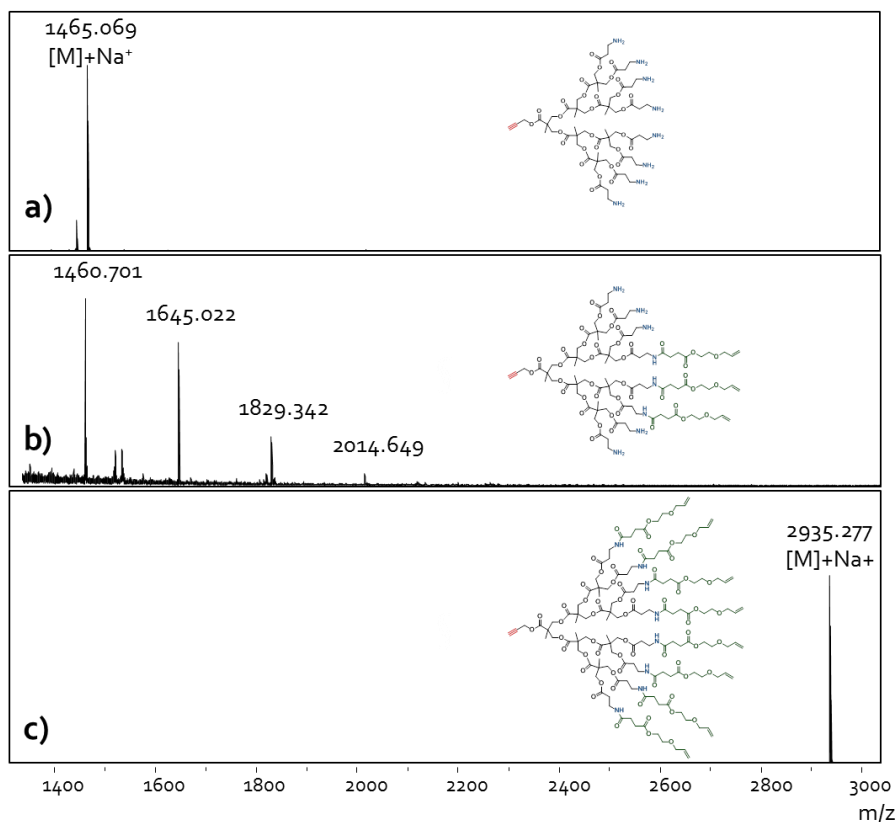


Figure 3: MALDI-TOF MS spectra of a) starting dendron (PFd-Acetylene-G₃-NH₂), b) dendron during a reaction and c) dendron after reaction completion

Note for reaction in water: The primary amine and NHS ester reaction is very efficient around physiological pH. However, at higher pH conditions, the hydrolysis of NHS ester can compete with the amine-NHS reaction. We recommend use high excess of NHS-derivatives in such cases.

Protocol (with water as solvent)

- Dissolve the dendron/dendrimer (1 equiv.) and NHS-derivative (1.5 ≥ equiv. per amine) in deionized water at a concentration of 50 mg/mL.
- Add NaHCO₃ (0.8 mg/mg of dendrimer or dendron). Stir the reaction at room temperature for 1 hour.
- Follow the reaction by MALDI-TOF MS. The increase in molecular weight can be observed where each peak corresponds to the attachment of NHS derivatives to the dendron. The MALDI-TOF MS spectrum will show the monodisperse peak at the final molecular weight, figure 2c.
- Dilute with water and purify by dialysis (this may not be possible with lower molecular weight products).
- The product can be isolated *via* freeze-drying.

Reference

- P. Stenstrom, M.Malkoch *et al* "Synthesis and in Vitro Evaluation of Monodisperse Amino-Functional Polyester Dendrimers with Rapid Degradability and Antibacterial Properties" *Biomacromolecules*, **2017**, *18* (12), pp 4323–4330.

Protected amine bis-MPA dendrons available from Polymer Factory

Product name	Generations available (<i>n</i>)	Functional groups	
		Core	End group
PFd-Gn-NHBoc-Azide	1 - 4	NHBoc	Azide (2 - 16)
PFd-Gn-NHBoc-Acetylene	1 - 4	NHBoc	Alkyne (2 - 16)
PFd-Gn-NHBoc-COOH	1 - 4	NHBoc	COOH (2 - 16)
PFd-Gn-Azide-NHBoc	1 - 4	Azide	NHBoc (2 - 16)
PFd-Gn-Acetylene-NHBoc	1 - 4	Alkyne	NHBoc (2 - 16)
PFd-Gn-COOH-NHBoc	1 - 4	COOH	NHBoc (2 - 16)
PFd-Gn-Thiol-NHBoc	1 - 4	Thiol	NHBoc (2 - 16)

Protected amine bis-MPA dendrimers available from Sigma-Aldrich

Product name	Generation	End group functionality
PFD-G1-TMP-NHBoc bis-MPA-NHBoc dendrimer, trimethylol propane core, generation 1	1	NHBoc (6)
PFD-G2-TMP-NHBoc bis-MPA-NHBoc dendrimer, trimethylol propane core, generation 2	2	NHBoc (12)
PFD-G3-TMP-NHBoc bis-MPA-NHBoc dendrimer, trimethylol propane core, generation 3	3	NHBoc (24)
PFD-G4-TMP-NHBoc bis-MPA-NHBoc dendrimer, trimethylol propane core, generation 4	4	NHBoc (48)

Disclaimer

The coupling reaction between primary amines and NHS esters are well established and are robust and thoroughly investigated by the scientific community. However, these protocols are intended to serve as a guide for your own research, and are not guaranteed to work with all substrates.