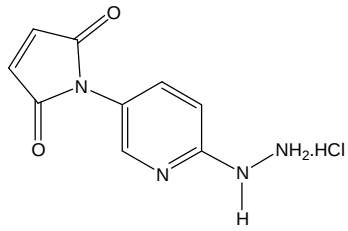
MHPH

$C_9H_8N_4O_2$.HCl, MW 240.65
Catalog# S-1009



MHPH (Solulink catalog:
S-1009) is a maleimide that converts thiols on biomolecules and surfaces to HyNic linker molecules for conjugation to 4FB-modified biomolecules.

Introduction to Solulink Bioconjugation Technology

Solulink's core technology is based on the formation of a stable aromatic bond formed from an aromatic hydrazine and an aromatic aldehyde. S-HyNic **1** (succinimidyl 6-hydrazinonicotinate acetone hydrazone) is used to incorporate aromatic hydrazine linkers on biomolecules. S-HyNic is an amino-reactive reagent that directly converts amino groups on biomolecules and surfaces to HyNic groups. S-4FB **2** (succinimidyl 4-formylbenzoate, SFB) is used to convert amino groups to aromatic aldehydes (4-formylbenzamide (4FB) groups). Addition of a HyNic-modified biomolecule to a 4FB-modified biomolecule or surface directly leads to the formation of the conjugate (Figure 1). The conjugate bond is stable to 92^{O}C and pH 2.0-10.0. The recommended pH for antibody conjugation is 6.0. Unlike thiol-based conjugation protocols where reducing reagents are required that that can compromise the activity of proteins by cleaving disulfide bonds, the HyNic-4FB conjugation couple leaves disulfide bonds intact. No oxidants, reductants or metals are required in the preparation of conjugate.

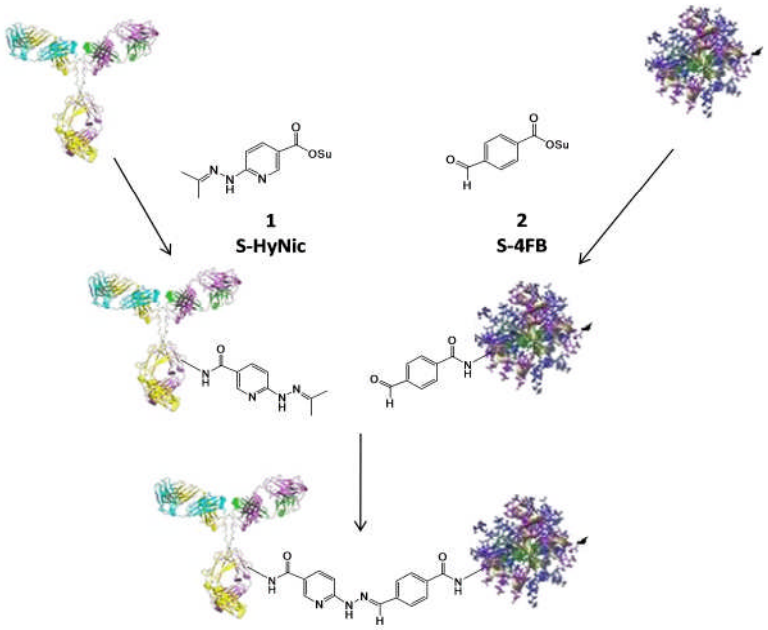


Figure 1: Schematic representation of SoluLinK Bioconjugation chemistry where an antibody is modified with S-HyNic to incorporate HyNic groups and a second protein is modified with S-4FB to incorporate 4FB groups. Conjugate is formed directly by simply mixing the HyNic-modified antibody with the 4FB-modified proteins.

Further enhancing the many advantages of the HyNic/4FB conjugation couple is the discovery by Dirksen *et al.*[1] that showed that aniline catalyzes the formation of this Schiff's base. This is especially effective for large biomolecule conjugations. In the case of antibody-protein conjugations the addition of 10 mM TurboLink Catalyst Buffer (aniline) to the reaction mixture converts >95% of the antibody to conjugate in ~2 hours using 1-2 mole equivalents of second protein.

The HyNic-4FB conjugation couple is chromophoric- the conjugate bond absorbs at 354 nm and has a molar extinction coefficient of 29000. This allows (1) real time spectrophotometric monitoring of a conjugate reaction, (2) ability to 'visualize' the conjugate during chromatographic purification using a UV or photodiode array detector and (3) quantification of conjugation. Furthermore, the level of incorporation of HyNic groups can be quantified colorimetrically as reaction with 2-sulfobenzaldehyde (Solulink catalog# # S-2005-100) yields a chromophoric product that absorbs at A350 with a molar extinction coefficient of 20000 (Figure 2). Links to procedures and calculators to guide users through this process are given in the procedures below.

Methods

Additional Materials Required

Reagents

Diafiltration spin columns	S-4004
Modification Buffer	S-4003
Conjugation Buffer	S-4002
DMF (anhydrous)	S-4001
2-sulfo-benzlaldehyde (S-2005)	
10mM TCEP·HCl in water (MW 286.65)	

Equipment

Variable-speed bench-top microcentrifuge
Spectrophotometer or Plate Reader
1.5 mL microcentrifuge tubes

Note: This protocol and all documents linked below can be downloaded from the appropriate category in the Solulink Library at http://www.solulink.com/library.

Modification Procedure

A. Desalting procedure

1. Desalt/buffer exchange the protein or oligonucleotide into Modification Buffer (100 mM sodium phosphate, 150 mM sodium chloride, pH 8.0); if needed, refer to the **Protein Desalting Protocol** or **Oligonucleotide Desalting Protocol**, if needed.

Notes:

a) High-level buffering capacity, *i.e.* 100 mM phosphate, is necessary for successful modification.

b) For desalting proteins Solulink recommends Pierce Zeba Desalt Spin columns (# 89882); for oligonucleotides, Sartorius Vivaspin diafiltration units (#VS0112). Refer to desalting protocol for either apparatus.

B. Determine the concentration of the protein

1. Determine the concentration of the protein to be modified using a **Bradford assay** (BioRad, #500-0006) or the **BCA assay** (ThermoScientific, #23223). Alternatively the A280 can be used if the protein extinction coefficient is known (E1%).
2. Adjust the concentration to 1-2.5 mg/mL in Modification Buffer pH 8.0, if necessary.

C. Prepare a MHPH/DMF stock solution

1. Prepare a stock solution of MHPH in anhydrous DMF (or DMSO) by dissolving 2-4 mg of MHPH in 100 µL anhydrous DMF.

Note:

a) The MHPH/DMF stock solution is stable for 2 weeks if prepared with anhydrous DMF (Solulink catalog# S-4001) and stored desiccated.

IgG concentration	MHPH molar equivalents added	Determined ratio of HyNic/protein (pH 8.0)
1.0	20	5.3
	30	7.8
4.0	15	4.6
	20	6.1
	25	7.8

Table 1: The number of HyNic groups incorporated on an antibody is dependent on the number of mole equivalents MHPH added and the protein concentration.

D. Modification of a protein or oligonucleotides

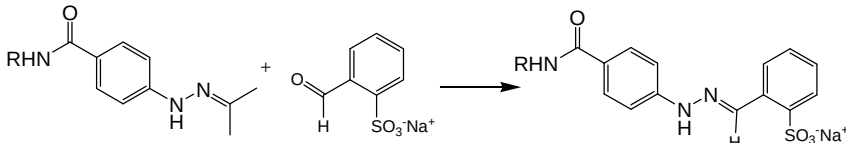
1. In most cases, a reduction step is required to break disulfide bonds prior to modification with MHPH. To do this, add 1/10th volume of 10 mM TCEP·HCl in molecular grade water to the buffer-exchanged protein; If the protein being modified already contains free thiols, this step may not be required.
2. Using Table 1 as a guide, add the requisite volume of MHPH/DMF to the protein solution; refer to the **Protein Modification With An NHS Ester Calculator** or **Amino-Oligonucleotide Modification With An NHS Ester Calculator**, if needed.
   a. Note: be sure to use the correct values for MHPH in the ***Reagent Information*** section of the calculator
3. Allow reaction to incubate at room temperature for 2.0 hours.

E. Desalting procedure

1. Desalt/buffer exchange the protein or oligonucleotide into Conjugation Buffer (100 mM sodium phosphate, 150 mM sodium chloride, pH 6.0); if needed, refer to the **Protein Desalting Protocol** or **Oligonucleotide Desalting Protocol**, if needed.

F. Quantifying molar substitution ratio

1. The molar substitution ratio (MSR) can be determined using a colorimetric reaction outlined in Figure 2. Addition of 2-sulfobenzaldehyde to a HyNic-modified biomolecule yields a bis-arylhydrazone that absorbs at 350 nm. Refer to the **HyNic-Protein Colorimetric MSR Calculator** or the **HyNic-Oligonucleotide Colorimetric MSR Calculator** as well as the protocol that is appropriate for your lab equipment: **HyNic Colorimetric MSR Assay Protein Nanodrop Method Protocol**, **HyNic Colorimetric MSR Assay Protein Spectrophotometer Method Protocol**, **HyNic Colorimetric MSR Assay Oligonucleotide Nanodrop Method Protocol**, or **HyNic Colorimetric MSR Assay Oligonucleotide Spectrophotometer Method Protocol**.



2. The biomolecule is now HyNic-modified and ready for conjugation to 4FB-modified biomolecules and surfaces.

Troubleshooting

Problem	Possible Cause	Solution
Protein was not modified or poorly modified.	Protein has been contaminated with amine containing compounds	Desalt the protein more thoroughly with a new Zeba Spin column or VivaSpin diafiltration device
	The concentration of the protein was too low	Increase the concentration of the protein to >2.0 mg/mL
MHPH was hydrolyzed	Wet or poor quality DMF/DMSO hydrolyzed the NHS ester	Use a good quality anhydrous DMF/DMSO to solubilize the MHPH molecule.

Stability

The stability of HyNic groups on proteins and other biomolecules varies. **It is recommended to conjugate HyNic-modified biomolecules immediately.**

Related Solulink Products

S-9002-1	S-HyNic Kit	S-4004	VivaSpin diafiltration device	S-2005	2-Sulfobenzaldehyde
S-1037	SS-S-4FB Linker	S-4001	DMF anhydrous	S-2006	TurboLink Catalyst Buffer

S-4000 Modification Buffer S-4002 Conjugation Buffer

References

1. Dirksen, A., et al., Nucleophilic catalysis of hydrazone formation and transimination: implications for dynamic covalent chemistry. J Am Chem Soc, 2006. 128(49): p. 15602-3.
2. Igor A. Kozlov, Peter C. Melnyk, Katie E. Stromsborg, Mark S. Chee, David L. Barker, Chanfeng Zhao, Efficient strategies for the conjugation of oligonucleotides to antibodies enabling highly sensitive protein detection, Biopolymers 2004, ***73***, 621.
3. Ryan C. Bailey, Gabriel A. Kwong, Caius G. Radu, Owen N. Witte, and James R. Heath, DNA-Encoded Antibody Libraries: A Unified Platform for Multiplexed Cell Sorting and Detection of Genes and Proteins, J. Amer. Chem. Soc. 2007, **129**, 1959-1967.