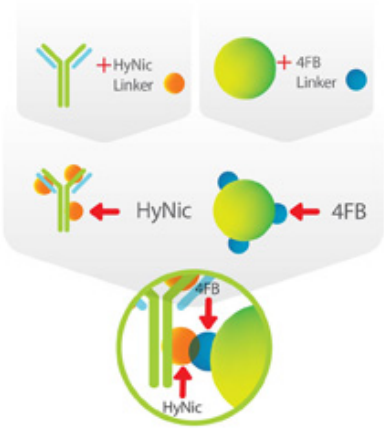
Hydrazine chemistry reagents
SANH/SHNH/SHTH, MHPH/MTFB, SFB

Introduction to Hydrazine chemistry conjugation



The hydraLinkk chemistry use 2 aromatic moieties, HyNic and 4FB, that react readily to yield a stable hydrazine bound. These moieties are previously separately grafted to biomolecules to be conjugated, while stable and prone to quantification.
Furthermore, this hydrazine chemistry adds other advantages over standard coupling chemistries, homofonctionnal ones like glutaraldehyde method, and heterobifunctionnal ones like the popular succinimidyl-malemide (NHS-MAL, SMCC) method.

➢ **Labeling, conjugation**
➢ **Immobilisation** on supports

Advantages : *so efficient and flexible conjugation !*

- **Apply to any kind of molecules and supports:**
  .proteins, peptides, antibodies, enzymes,… Glycosides, Lipids,..
  .nucleotides, nucleic acids,.. Supports: beads, agarose, …
- **easy** operating (modify each molecule, mix),
- **no reduction, oxidation, metals or deprotection step**,
- **stable activated biomolecules** - for months,
- **controllable reaction and activation levels** - by colorimetric monitoring/dosage
- **better control** of the coupling ratio,
- **excellent yield** of conjugation,
- **tracable**! control the formed coupling bond by absorbance at 354nm
- **very stable formed bond** - to 92°C and pH 2-10,
- heteroconjugation is **highly selective and orientated** (specific),
- conjugation keep the bioactivity of the components, non-interfering (bioorthogonal)
- **Options** – using available **cleavable** linkers, **extended C6** and **hydrophilic PEO$_4$** spacers
- A complete range of **HyNic and 4FB reagents** (amine or sulfhydryl reactive, spacer,…)

The hydraLink potentiates the Hydrazine chemistry by the adjunction of aromatic groups, forming a stable bis-aryl hydrazone-conjugate.

➲ One biomolecule is modified to yield a derivate bearing a hydrazinopyridine group (HyNic,  -Hydrazone)

SHNH allows to couple a hydrazinopyridine group on amine (lysines), through reaction of an NHS ester (acylation, see below).

SANH performs similarly but with hydrazine protection. The protecting group leaves during formation of the hydrazone conjugate, making it useful as standard reagent. The **C6-SANH** reagent has an extended 6 carbon spacer, for lower steric hindrance.

MHPH allows to couple a hydrazinopyridine group on sulfhydryls (Cysteines), thought reaction of a maleimide (see below).

SHTH #BL9370 yields an aromatic hydrazide group, while **SATH** #BL9390 a 4-hydrazidoterephtalamide group.

➲ The second biomolecule to be conjugated is modified to yield a derivate bearing an aldehyde group (FB, |-CHO).

SFB allows to couple an aldehyde group on amine (lysines), thought reaction of an NHS ester (acylation, see below). **SulfoSFB** is a water soluble analogue. The **C6-SFB** reagent has an extended 6 carbon spacer, for less steric hindrance.

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MTFB allows to couple an aldehyde group on sulfhydryls (Cysteines), thought reaction of a maleimide (see below).

➲The 2 derivatized ligands are stable (can be stored for months, i.e. frozen) and their activative groups (hydrazinopyridine and aldehyde) can be monitored using a colorimetric reaction with 4NBA #BL96540 and 2HP #O19022 respectively. HyNic and FB groups will react readily together once mixed. The coupling ratio can be thus be strictly controlled, and more accurately for several experiments needed to development of the right conjugate.

More information is given further in the section 'Additional Information'. Selecting right reagent/application

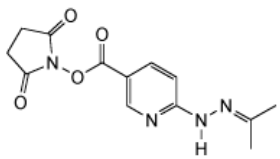
Select the right reagent depending on desired conjugate:

Type of Conjugate	molecule1	Target Reactive Group1 =>use product as Linker	Molecule2	Target Reactive Group2 =>use product as Linker
Antibody / Protein	Ab	Amino (NH2) =>SANH	Protein	Amino (NH2) =>SFB
		Thiol (SH) =>MHPH		Thiol (SH) =>MTFB
Protein / Oligo	Protein	Amino (NH2) =>SANH	Oligo	3' or 5' Amino =>SFB
		Thiol (SH) =>MHPH		dNTPs =>4FB-phosphoramidite
Protein / Peptide	Protein	Amino (NH2) =>SANH	Peptide	N-terminus =>BOC-HyNic
		Thiol (SH) =>MHPH		C-terminus =>6-FMOC-Lys-tBoc-HyNic
Oligo / Peptide	Oligo	3' or 5' Amino =>SFB, or sulfo-SFB, or SS-S-4FB (cleavable)	Peptide	N-terminus =>BOC-HyNic
		dNTPs =>4FB-phosphoramidite		C-terminus =>6-FMOC-LystBoc-HyNic
Protein / Supports	µsphere Beads Agarose Beads	=>4FB NanoLink Beads or 4FB-Agarose	Ab, other protein, rPE, APC, perCP, HRP, AlkPhos,…	Amino (NH2) =>S-HyNic
				Thiol (SH) =>MHPH
Peptide / Supports	µsphere Beads Agarose Beads	=>4FB NanoLink Beads or 4FB-Agarose	Peptide	N-terminus =>BOC-HyNic

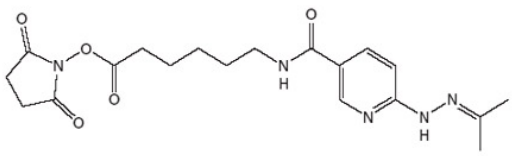
Product Information

Incorporation of aromatic hydrazinopyridine and aldehyde moieties on proteins or other biomolecules

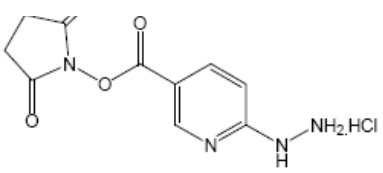
Catalog #: BL9270, 10 mg BL9271, 25 mg
Name: **SANH (S-HyNic)**
Succinimidyl 6-hydrazinonicotinate acetone hydrazone
MW 290.2 (M)
Used to convert amines to hydrazinopyridine moieties



Catalog #: BL9330, 10 mg
Name: **C6-SANH**
C6-Succinimidyl 6-hydrazinonicotinate acetone hydrazone
MW 403.44 (K)
6 carbon spacer analog of SANH



Catalog #: BL9360, 10 mg
Name: **SHNH**
Succinimidyl Hydraziniumnicotinate Hydrochloride
MW 286.68 (M)
Used to convert primary amines to hydrazinopyridine moieties. Also chelates 99mTc.



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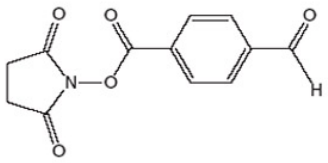
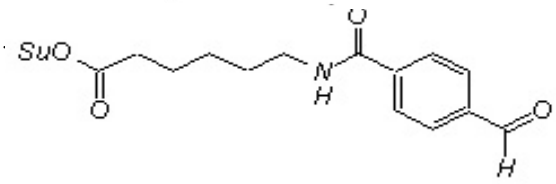
Catalog #: BL9370, 10 mg
Name: **SHTH**
Succinimidyl 4-Hydrazidoterephthalate.Hydrochloride
MW 313.7 (M)
Used to convert I amines to HyNic moieties.

Catalog #: BL9390, 25 mg
Name: **SATH**
Succinimidyl 4-hydrazidoterephthate acetone hydrazone
MW 317.4
6 carbon analog of SANH
Used to incorporate 4-hydrazidoterephthalamide moieties on proteins or other amine containing moieties. This is a custom product ; please call for availability.

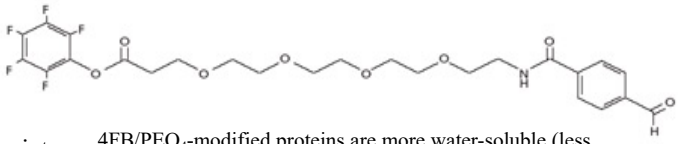
Catalog #: M11771, 100 mg
Name: **SFB (S-4FB)**
Succinimidyl-4-formylbezoate
(M) CAS: [60444-78-2]; MW 247.21 (K)
Used to convert amines to benzaldehyde moieties.



Catalog #: BL9410, 25 mg
Name: **C6-SFB**
C6-Succinimidyl-4-formylbezoate
MW 336.35 (K)
Extended-spacer version of SFB
Used to convert primary amines to benzaldehyde moieties

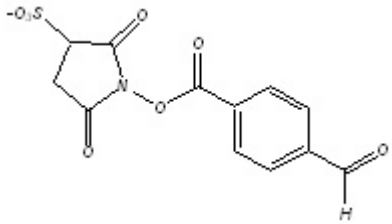


Catalog #: CP3981, 10 mg
Name: **PEO_4-SFB (PEG_4-SFB)**
(K) MW 563.5 (K) desiccated
PEO spacer version of SFB.
The extended PEG linker enhances solubility and alleviates steric hindrance with increase in conjugate yield.

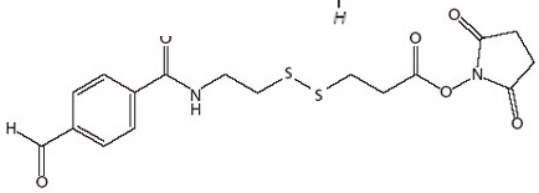


4FB/PEO_4-modified proteins are more water-soluble (less hydrophobic) than 4FB-modified proteins, which can eliminate precipitation issues with certain proteins.

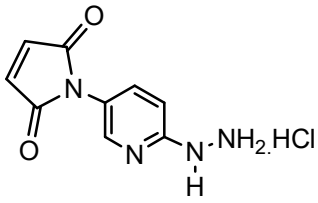
Catalog #: BI1311, 25 mg
Name: **Sulfo-SFB (sS-4FB)**
Sulfo-Succinimidyl-4-formylbenzoate
MW 327.27 (K) desiccated
Water-soluble version of SFB.
Especially useful for conversion of amino surfaces such as beads and plates as this reagent is water soluble.



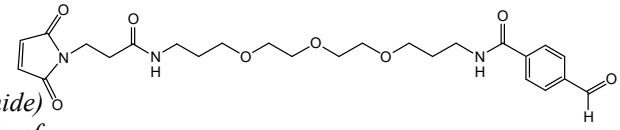
Catalog #: CP3991, 10 mg
Name: **SS-SFB (SS-S-FB)**
Succinimidyl-4-formylbezoate
(K) MW 410.5 (K) dessicated
Thiol mediated cleavable version of C6-SFB. Useful for intracellular conjugate cleavage / release in cytoplasm.



Catalog #: BL9400, 10 mg
Name: **MHPH (Mal-HyNic)**
3-N-Maleimido-6-hydraziniumpyridine hydrochloride
(M) MW 290.2 (M)
Used to convert thiols to hydrazinopyridine moieties.



Catalog #: BZ0774, 5x1 mg
Name: **MTFB (Mal-PEO_3-HyNic)**
MW 503.54 (M)
Used to convert thiol moieties to 4FB (4-formylbenzamide) moieties. Possess a PEO3 linker for increased solubility of modified biomolecule.



Note: Store under inert atmosphere), dessicated, at +4°C (or frozen/long term)(K), or frozen (M)
For research use only. Not for internal or external use in humans.
Most products are available in 5x1mg size and bulk quantities.

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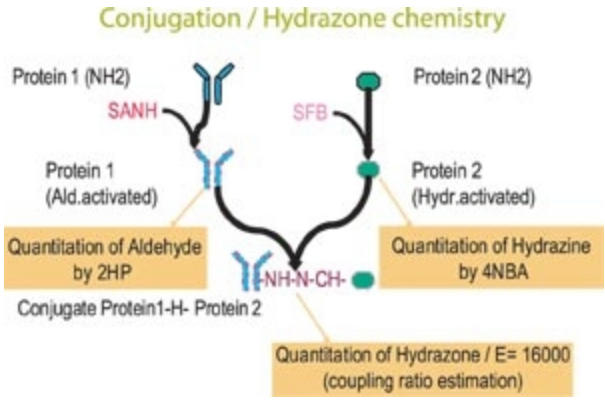
Directions for use

The following is information and guidelines for use suit notably to conjugate proteins (or amine containing molecules such aminoallyl nucleotides, or supports). Typically, a protein 1 is activated using SANH reagent (NHS-HyNic), while the other is modified by SFB reagent (NHS-CHO).
Activation levels can be controlled using a colorimetric dosage with 2HP and 4NBA reagents.
Activated proteins are then simply mixed allowing conjugation.



Guidelines for use – protein activation to bear HyNic, HNA or 4FB reactive groups

Required reagents and equipment:
Diafiltration spin columns
Modification Buffer
Conjugation Buffer
DMF (anhydrous)
2-sulfo-benzaldehyde
Variable-speed bench-top microcentrifuge
Spectrophotometer or Plate Reader
1.5 mL microcentrifuge tubes

See related products, and also additional information.

Protocol for NHS derivatisation: SANH or SHNH or SFB activation step to yield a HyNic or HNA or 4FB moiety

1. **Preparer the protein in suitable buffer at 1-5mg/ml**

The protein (or amine containing molecule or support) should be in amine-free and sulfhydryl buffer at pH 7.3 to 9. Alternatively, adjust the pH, or perform buffer exchange by dialysis or desalt by another mean, to remove Tris or free aminoacids (glycine). High-level buffering capacity, i.e. 100 mM phosphate, is recommended.
The concentration of the molecule to activate should be high enough to get efficient yield.

Amine content can be determined by OPA colorimetric of fluorimetric dosage using OPA reagent.
Protein content can be determined using BC Assay or Coo Assay (for oligonucleotides, use 260nm absorbance)

Typically prepare an antibody or peptide at 2.5mg/ml concentration in PBS pH7.6 or Carbonate pH 8.3.

1. **Prepare a NHS modifier solution** (SFB, SANH or SHTH) at 20-100mM.

I.e. dissolve 2-4mg of SANH or SHNH or SFB in 100µl DMF

2. **Add the desired quantity of modifier solution to the protein**:

- Put sufficient **quantity of protein** to take in consideration protein loss in the desalting step..
- Determine from below tables the **molar ratio of NHS modifier to protein** required to get desired coupled ratio.

A typical condition is 10 molar equivalents of modifier for a 2mg/ml protein solution. Hence, to 2mg of antibody (1ml at 2mg/ml = 12.5µM), add 125µM of NHS modifier, that is 12.5µL of a 100mM solution of SANH (29.0mg/ml) or of SFB (24.7mg/ml).
- Keep <3-10% DMF if the biomolecule is sensitive to organic solvents.
- We recommend optimizing the modification conditions for each new protein, as the over-modification may result in precipitation, or loss a biological activity.

IgG concentration (mg/mL)	SANH or SHTH molar equivalents added	Determined ratio (MSR) of SANH or SHTH/protein or oligo
1.0	20	5.5
	30	8.2
4.0	15	4.7
	20	6.4
	25	7.8

Equivalents of SFB/IgG	mg/ml	MSR
0	0.325	0
5	0.414	1.23
10	0.321	4.12
20	0.301	5.7
30	0.283	8.61
40	0.272	11.6
50	0.260	13.7

The degree of substitution by Benzaldehyde was determined on goat IgGs with 0. 5. 10, 20, 30, 40, 50 equivalents of SFB. The UV absorbance of IgG between 300 and 350 nm is not significantly changed

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3. **Incubate at room temperature for 2 hours.**
Shorter incubation work, but may require a quenching step to get reproducible results.
Incubating at +37°C speed the reaction, but also speeds the hydrolysis of the NHS reactive group.

4. **Dessalt activated biomolecule** using a gelfiltration column, diafiltration or dialysis.
Please refer to desalting tool supplier for operating procedure.
For a next conjugation, use suitable buffer: 100 mM sodium phosphate, 150 mM sodium chloride, pH 6.0.
The 4FB linker molecules incorporated on biomolecules are stable at 4°C for >30 days.

5. **Determine the protein concentration** using a colorimetric method
such as BC Assay (UP40840) or Bradford Assay (UPF4600) or any other suitable assay.
Determine the HyNic (or 4FB) content by colorimetric dosage using 4NBA (or 2HP) – see below.

Troubleshooting for protein activation by NHS modifiers

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 desalting column
	The added quantity of modifier was too low	Check for calculations.
	The concentration of the protein was too low	Increase the concentration of the protein to >2.0 mg/mL
NHS was hydrolyzed in the modifier (SANH, SHTH; SFB)	Wet or poor quality DMF/DMSO hydrolyzed the NHS ester	Use a good quality anhydrous DMF/DMSO to solubilize the modifier (SANH, SFB, SHTH).
Too high modification level	The added quantity of modifier was too high and made modified protein unstable.	Check for calculations. Check for presence of aggregates, protein loss (assay before/after each step).

Protocol for MAL derivatisation: MHPH or MTFB activation step to yield a HyNic or 4FB moiety
The molecule to be derivatized to yield a HyNic or 4FB moiety should be in a sulfhydryl-free buffer at pH 7.3 to 9. Perform buffer exchange by dialysis or desalt by another mean in case DTT or other reducers are present. Buffers containing Tris or Glycine are also not recommended.
Typically prepare an SH-containing antibody or peptide at 1-5mg/ml concentration in Conjugation Buffer (0.1M sodium phosphate, 0.15M sodium chloride, pH 6.0)

1. **Prepare a MAL modifier solution** (MHTH or MFTB)
Dissolve 2-4mg of MHPH in 100µl of anhydrous DMF.
The MHPH/DMF stock solution is stable for 2 weeks if prepared with anhydrous DMF and stored desiccated.

2. **Mix protein solution and MHPH or MTFB solution** in desired molar ratio,
and incubate for 2hours.
Suitable ratio of MHPH for antibody activation:

IgG concentration	MHPH molar equivalents added	Determined ratio of HyNic/protein (pH 7.4)
1.0 mg/ml	20	5.3
	30	7.8
4.0 mg/ml	15	4.6
	20	6.1
	25	7.8

5. **Determine the protein concentration** using a colorimetric method
such as BC Assay (UP40840) or Bradford Assay (UPF4600) or any other suitable assay.
Determine the HyNic (or 4FB) content by colorimetric dosage using 4NBA (or 2HP) – see below.

Troubleshooting – for protein activation by maleimide modifiers

Problem	Possible Cause	Solution
Protein was not modified or poorly modified.	Protein has been contaminated with sulfhydryls (or amines) containing compounds	Desalt the protein more thoroughly with a new gelfiltration column or VivaSpin diafiltration device
	The concentration of the protein was too low	Increase the concentration of the protein to >2.0 mg/mL

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MHPH was hydrolyzed	Wet or poor quality DMF/DMSO hydrolyzed the Maleimide groups	Use a good quality anhydrous DMF/DMSO to solubilize the MHPH molecule.

Protocols for monitoring HyNic or 4FB moieties

Protocol to quantitate hydrazinopyridine incorporation by 4-NBA #BL9650:
4-NBA (p-NBA) reagent must be protected from light.

1. Prepare a 0.5M 4NBA solution: Dissolve 4NBA in DMF, and add the required volume to Conjugation Buffer. The solution is stable for 1 month at+4°C when protected from light.

2. Add an aliquot of the Hydrazine-modified protein solution to the pNBA solution and incubate at +37°C for 1 hour, or at room temperature for 1 hours. Determine the absorbance at 390nm against a blank (equal aliquot of buffer + pNBA solution).

3. The **hydrazine/protein MSR = Abs_{380nm} / 20 000 / [protein concentration in mol/L]**
where A380nm: absorbance at 380nm (1cm path), to estimate hydrazine concentration (EC:20 000)
[Protein M]: protein concentration in assay sample in M, as dosed in step 5.,
or calculate as = [protein concentration in mM] x (used volume in ml) / (total assay volume in ml)

Protocol to quantitate 4FB aldehyde reactive groups incorporation by 2HP #019022 :
2HP reagent must be protected from light.

1. Prepare a 0.5mM 2HP solution in 100mM MES pH4.7 buffer
Dissolve 2HP in DMF, and add the required volume to Conjugation Buffer. The solution is stable for at least 1 month at +4°C when protected from light.
Other buffers may need to be used if protein precipitation occurs due to the pI of the protein. For example, Citrate buffer (pH 5-6) can be used in place of MES (4.7).

2. Add an aliquot (10µl) of the benzaldehyde-modified protein sample (1-5mg/ml) to the 2HP solution (490µl 0.5mM) and incubate at +37°C for 1 hour, or at room temperature for 2 hours. Determine the absorbance at 350nm against a blank (equal aliquot of buffer + 2HP solution).

3. Calculate the number of aldehyde moieties/protein (MSR: Molar Substitution Ratio [protein]/[Hydrazone]):
The **aldehyde/protein MSR = Abs_{350nm} / 24 500 / [protein concentration in mol/L]**
where A350nm: absorbance at 350nm (1cm path), to estimate hydrazone concentration (EC:24 500)
[Protein M]: protein concentration in assay sample in M, as dosed in step 5.,
or calculate as = [protein concentration in mM] x (used volume in ml) / (total assay volume in ml)
Note: if the number of reactive 2-hydrazinopyridine moieties on the biomolecule is a problem for quantitation, because of biomolecule precipitation for instance, uses a solution of SBF 0.5mM in Conjugation buffer instead of pNBA (follow the same protocol).

Guidelines for use – protein conjugation

Bring the concentration of activated proteins to 1-5 mg/mL in buffer pH 6.0 for the conjugation step.

1. **Mix the activated the hydrazine-protein1** (SANH-, SHTH-, or MHPH-activated) **and benzaldehyde-protein2** (SFB- or MTFB-activated).
-for proteins: at a 1:1 to 1:3 molar ratio –eventually higher (1:10) -depends on protein size and desired features-,
-for oligonucleotides: at a 1:1.5 molar ratio

Note: please refer to section 'Technical and Scientific Information' of kit #BL150 for specific applications (high MW conjugate preparation)

2. **Incubate for 2-16 hours at room temperature** in Conjugation buffer.
.*Note*: The reaction can be speeded using 1-10mM TurboCatalys reagent (see additional info). This is especially useful for large proteins.

.*Note*: The extent of conjugation can be monitored according step 4. Alternatively, determine it on an aliquot of the reaction mixture by SDS-PAGE analysis of a protein-protein conjugate, or by PAGE analysis for protein-oligonucleotide conjugate.

.*Note*: In case excess of hydrazinopyridine moieties remain unreacted on the biomolecule, and not desired, they can be quenched by using a solution of 2-SBA (#A42050) at 0.5mM in Conjugation Buffer for 2 hours.
An alternative method is desalting by suitable mean.

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3. **Desalt the conjugate**. The conjugate can be isolated by standard size exclusion chromatography (gelfiltration columns UP84874), diafiltration (UptiSpin), or dialysis (CelluSep). For purer conjugates, we recommend FPLC to isolate protein-protein, and size exclusion for protein-oligonucleotide conjugates.

4. **Controlling the conjugation level**:

The HyNic-4FB conjugation bond is chromophoric: the conjugate bond absorbs at 354 nm and has a molar extinction coefficient of 29 000. 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,
(3) quantification of conjugation.
.*Note*: the absorbance measurement may be ruined in case the biomolecules or the used buffer absorb at 354nm.

Additional information

●NHS & Maleimide reactions steps – for protein "activation"

NHS-ester derivatives (SANH, SHTH, SFB) are suited for direct labeling of amino groups in proteins (typically Lysine residues) and aminated DNA/RNA (i.e aminoallyl nucleotides labeled materials).
The chemical group N-hydroxysuccinimidyl (NHS) reacts optimally at neutral pH or higher in aqueous phase on primary (–NH2) and secondary amines (-NH-) (in fact on its deprotonated form): i.e. amines present in proteins (Lys amino-acid) and in a lower proportion on NH2 located in terminal peptidic chains. The reaction competes with hydrolysis that increases with pH, and with the high dilutions of the molecule that should be labeled. Please refer to the literature, or the technical sheet FT-BA6800 (NHS-FluoProbes labels) for a standard protein coupling protocol.

Maleimide derivatives (MHPH, MTFB) are suited for labeling of thiol groups of proteins or other molecules, e.g. specific labeling of cysteine. Please refer to the literature, or the technical sheet FT-BA6810 (Maleimide-FluoProbes labels) for a standard protein coupling protocol.

The proteins to be conjugated should be prepared in suitable **buffer** (devoid of amine or sulfhydryls).

A **quenching** step to neutralize un-reacted NHS (i.e. by lysine) or Maleimide agents (i.e.by cysteine), or a desalting step to remove them, may be required if one of the activated molecule may be modified by the excess reagent of the other activation step. It is also needed when checking for the activation level (HyNic, or 4FB quantitation)

Determine the **concentration** of the protein to be modified using the BCA assay (UP40840A) or spectrophotometrically if the extinction coefficient of the protein is known. The extinction coefficient for antibodies is ~1.4-1.6 for a 1 mg/mL solution.

●HyNic/4FB conjugation

The recommended **pH** for antibody conjugation is 6.0. The conjugate bond is **stable** to 92°C and pH 2.0-10.0.

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.

The HyNic/4FB reaction can be **catalyzed** but the TurboLink Catalyst (1). This is especially effective for large biomolecule conjugations. In the case of antibody-protein conjugations the addition of 10 mM TurboLink Catalyst Buffer 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 (2NPA) yields a chromophoric product that absorbs at A350 with a molar extinction coefficient of 20000. See the above procedures to guide user.

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SANH (S-HyNic)

SANH reagent (S-HyNic, NHS-HyNic) is a Succinimidyl (NHS) ester that converts amines on biomolecules and surfaces to HyNic linkers for conjugation to 4FB-modified biomolecules, in a single step. Beside this modifier function, the introduced HyNic moiety (6-hydrazinonicotinamide) acts as a linker that forms stable covalent conjugates with biomolecules possessing 4FB (4-formylbenzamide) incorporated linkers. SANH is thus a heterobifunctionnal crosslinker for SH-containing molecules and 4FB-modified molecules.

SANH is most widely used to produce a variety of antibody-related conjugates such as IgG-HRP, IgG-AP and IgG-oligos. Conjugates of other antibody fragments have also been made (scFv, Fab, and F(ab)'). SANH is also used for the synthesis of numerous other conjugates including: general protein-protein, protein-peptide, peptide-oligonucleotide, enzyme-oligonucleotide, and DNA or RNA-protein conjugates. Bioconjugates made using SANH can be used in ELISA assays, Immuno-PCR, in situ detection (FISH), Westerns, Southerns, and in many other biological applications.

The advantages of the SANH-4FB linker system include a unique control to the entire conjugation process, thanks to:
●**reaction specificity for the activation step**: SANH readily reacts with primary amines on a protein (ε-amino group of lysine) via an NHS-ester, yielding a HyNic-activated molecule.

●**traceability**: SANH introduces UV-traceable HyNic groups (hydrazinonicotinate) into proteins or other biomolecules, bringing unique control to the entire conjugation process. Furthermore, it can be quantitate using 4NBA.

●**reaction specificity for the conjugation step**: the introduced HyNic group reacts only with aromatic aldehydes, even in the presence of -NH2, -SH, -COOH and other protein functionalities). Hence aminated molecule1 modified by SANH –i.e. a HyNic-modified peptide, antibody or oligos) reacts to form stable conjugates in the presence of aminated molecules2 modified by SFB (i.e.aromatic aldehyde-modified proteins or nucleic aminollyl acids).

Solubility: >50mg/mL in DMF.

See HyNic/4FB conjugation additional information.
protocols: protein activation and protein conjugation.

References - SANH
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.

SHTH

SHTH (BL9370) is homologous to SANH reagent. It is an aromatic hydrazide linker used to incorporate HTA (4-hydrazido-terephalamide) linkers onto biomolecules through their amino group via an activated ester (i.e. NHS). **HTA linkers form heat labile hydrazide-based hydrazones with aromatic aldehydes** such as 4FB (4-formylbenzamide).

Solubility: >50mg/mL in DMF.

See HNA/4FB conjugation additional information.
protocols: protein activation and protein conjugation.

References - SHTH
1. **Dirksen**, A., et al., Nucleophilic catalysis of hydrazone formation and transimination: implications for dynamic covalent chemistry. J Am Chem Soc, 2006. 128, 15602-3.

SFB (S-4FB)

SFB (succinimidyl-4-formylbenzoate, S-4FB 2) is used to convert amino groups to aromatic aldehydes 4FB (4-formylbenzamide groups). Addition of a HyNic-modified biomolecule to a 4FB-modified biomolecule or surface directly leads to the formation of the conjugate.
For amino-modified surfaces it is recommended to use sulfo-S-4FB.

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SFB is thus a heterobifunctionnal crosslinker for NH2-containing molecules and 4FB-modified molecules.
The advantages of the SFB-HyNic linker system include reaction specificity, UV-traceability, and the unique control it brings to the entire conjugation process.

Solubility: >50mg/mL in DMF.

References - SFB

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.

MHPH (Maleimide-HyNic)

MHPH (Maleimide HyNic) is a modifier that converts thiols on biomolecules (i.e. Cysteins) and surfaces to HyNic linker molecules, in a single step. The introduced HyNic moiety (6-hydrazinonicotinamide) acts as a linker that forms stable covalent conjugates with biomolecules possessing 4FB (4-formylbenzamide) incorporated linkers. MHPH is thus also a heterobifunctionnal crosslinker for SH-containing molecules and 4FB-modified molecules.
The advantages of the MHPH-4FB linker system include reaction specificity, UV-traceability, and the unique control it brings to the entire conjugation process.

Hydrazone chemistry catalyser

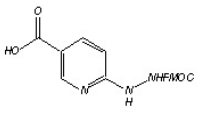
TurboLink Catalyst Buffer #HT1820
TurboLink Catalyst is used to catalyze the Hydralink bond formation reaction between 4FB modified biomolecules and HyNic modified biomolecules. It is added at 100-300 fold dilution, and the conjugation reaction is complete in 1-2 hours . This reagent is particularly useful for conjugations of large biomolecules; antibodies, proteins, enzymes, and surfaces.

Hydrazone chemistry colorimetric reagents

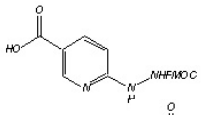
4NBA (or p-NBA) #**BL9650** (used to quantitate the level of hydrazide and hydrazine modification)
2HP #019022 (used to quantitate the level of aldehyde modification)
2SBA #**A42050** (used to cap hydrazinopyridine)
See protocols above.

Hydrazone chemistry reagents for organic synthesis

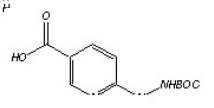
6-FMOC-HNA **BL9740, 100mg** **BL9741, 500mg**
6-FMOC-hydrazinonicotinic acid; MW: 375.2
Used to incorporate hydrazine moieties during solid or solution phase peptide synthesis.



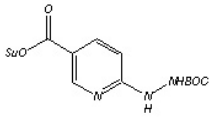
6-FMOC-HNA-Osu **BL9760, 100mg** **BL9761, 50**
Succinimidyl 6-FMOC-hydrazinonicotinate; MW: 472.2
Used to incorporate hydrazine moieties during solid or solution phase peptide synthesis.



6-BOC-HNA (BOC-HyNic) **BL9750, 100mg** **BL9751, 500mg**
6-BOC-hydrazinonicotinic acid; MW: 253.1
Used to incorporate hydrazine moieties during solid or solution phase peptide synthesis.

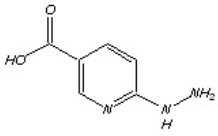


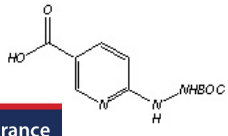
6-BOC-HNA-OSu **BL9770, 100mg** **BL9771, 500mg**
Succinimidyl 6-BOC-hydrazinonicotinate; MW: = 350.3
Used to incorporate hydrazine moieties during solid or solution phase peptide synthesis.



C6-HNAA **BL9780, 100mg** **BL9781, 500mg**
6-hydrazinonicotinic acid acetone hydrazone; MW: 306.4
Used to incorporate protected hydrazine moieties with extended six carbon linker during peptide synthesis

HNA **BL9790, 100mg**
6-Hydrazinonicotinic Acid; MW: 153.1
Precursor molecule.





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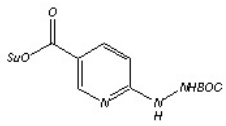
BOC-HNA **BL9810, 100mg** **BL9811, 500mg**

(4-BOC-hydrazido) terephthalic acid; MW: 280.3
Precursor molecule.

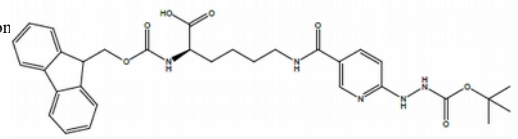
BOC-HTA-OSu **BL9820, 100mg** **BL9821, 500mg**

Succinimidyl 4-BOC-hydrazido) terephthalate; MW: 377.4
Precursor molecule.



FMOC Lysine-ε-(6-Boc-HyNic)OH (FMOC-Lysine-t-Boc-HNA) **JV3390, 100mg** **JV3391, 500mg**

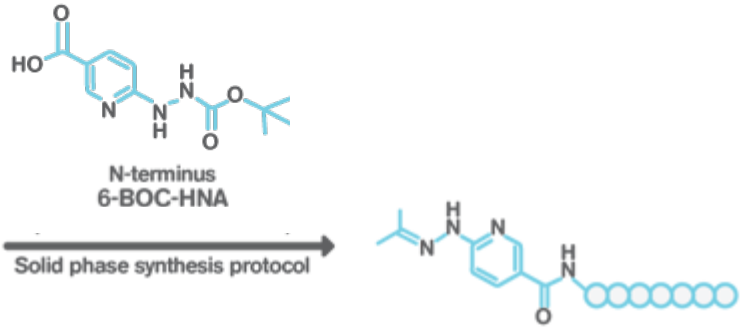
MW: 603.68
used to incorporate HyNic (6-hydrazinonicotinamide) moieties onto the C-terminus or internal position onto a small molecule, surface, or polymer with base-labile protection
Ideal for linking peptides to any biomolecule or surface to improve purification, detection, delivery, or targeting.



FEATURES LINKERS:

6-Boc-HNA

6 BOC HNA (6-BOC-hydrazinonicotinic acid) allows for peptide activation for linking peptides to any biomolecule or surface to improve purification, detection, delivery, or targeting.



6-BOC-HNA is used to incorporate BOC-protected HyNic (6-hydrazinonicotinamide) moieties onto peptides during solid or solution phase synthesis or onto a small molecule, surface, or polymer where acid-labile protection is required. It can be coupled to the N-terminal amino group as the last step in the synthesis, prior to cleavage, or coupled to the side-chain of a Lys residue which was introduced using an appropriately orthogonally protected derivative such as Lys(ivDde) or Lys(Mtt). The linker requires activation for incorporation during peptide synthesis. HyNic-modified peptides are readily conjugated to 4FB-modified biomolecules. Ask for paper: "A New Generation of Peptide Conjugation Products"

6-Fmoc-Lysine-t-Boc-HNA

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