Merck Biosciences

Calbiochem | Novabiochem | Novagen

Novabiochem®

HydraLinK™: Superior Technology for Bioconjugations and Immobilizations

Contents

HydraLinK™

HydraLinK™ is a novel bioconjugation system for the conjugation and immobilization of peptides, proteins, carbohydrates and DNA/RNA.

The technology is based on the reaction of a 2-hydrazinopyridyl moiety with a benzaldehyde moiety to yield a stable bis-aromatic hydrazone (Figure 1). The chemistry is highly selective, stable in solution and not susceptible to non-specific binding, making it superior to conventional methods of bioconjugation such as maleimide/thiol and avidin/biotin (Table 1).

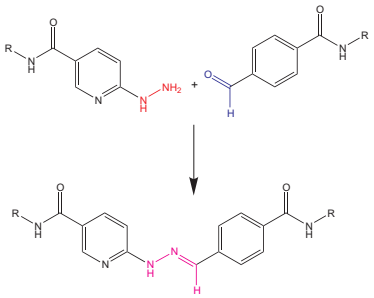
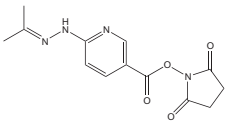


Figure 1: Chemoselective conjugation using the HydraLinK™ bioconjugation system.

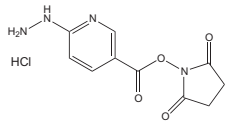
The technique can be readily engineered to link small molecules (peptides, fluorophores), biomolecules (proteins, DNA, RNA), or other molecules to solid surfaces (glass, plastic, latex, silica beads), for applications in proteomics, genomics, drug discovery, therapeutics and diagnostics.

	hydrazine/ carbonyl	avidin/ biotin	maleimide/ thiol
modified biomolecules stable over extended period	✔✔✔	✔✔✔	-
both moieties unreactive to all biomolecules	✔✔✔	✔✔	-
no undesirable covalent modification	✔✔✔	✔✔✔	-
no electrostatic/hydrophobic interaction	✔✔	-	-
formation of covalent (stable) linkage	✔✔	-	✔✔
stable under broad pH ranges	✔✔	✔✔	✔✔
fast reaction kinetics	✔✔✔	✔✔✔	✔✔
amenable to solid phase synthesis	✔✔✔	✔✔	-
stable surface modification	✔✔✔	✔✔	-

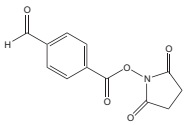
Table 1: Advantages of HydraLinK™.



SANH



SHNH



SFB

The implementation of the technology is extremely simple. Incorporation of the benzaldehyde moiety on proteins and surfaces is effected by treatment with SFB, succinimidyl 4-formylbenzoate. Similarly, introduction of the 2-hydrazinopyridine moiety is achieved using either SANH or SHNH. In SANH, the hydrazine group is protected as its acetone hydrazone; however, no separate deprotection step is required to free the hydrazine, as in mild acid the acetone rapidly exchanges with the aromatic aldehyde yielding the desired bis-aromatic hydrazone (Figure 2).

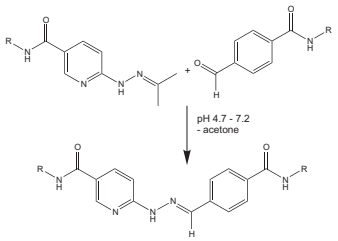


Figure 2: Conjugation of an SANH-modified biomolecule with an SFB-modified biomolecule.

Biomolecules modified with hydrazine and aldehyde modification reagents are then mixed to give the hydrazone-mediated conjugate. The leaving group in the reaction is water and no reducing reagents are required to stabilize the hydrazone. The reaction is optimally carried out at pH 4.7, but the reaction also occurs up to pH 7.3, albeit more slowly.

Advantages of the HydraLinK™ bioconjugation system

Enhanced stability

Enhanced stability of biomolecules modified with hydrazines and aldehydes leads to increased ease of use, reproducibility and efficiency. Figure 3 shows stability data of hydrazine-modified IgG. This extended stability allows modified proteins to be prepared days or weeks in advance of conjugation or immobilization and reaction conditions to be optimized prior to scale up. By contrast, with maleimido/thiol chemistry, modification and conjugation of both proteins must be done immediately in most cases.

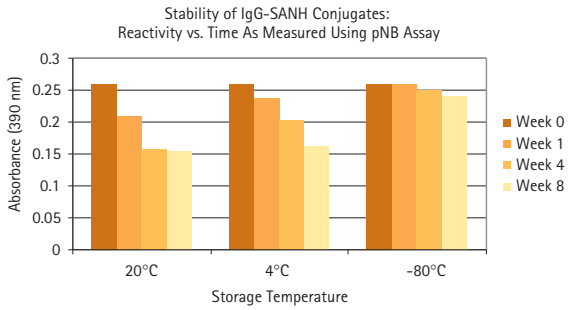


Figure 3: Stability of hydrazinopyridinyl moieties conjugated to IgG.

Non-denaturing

As no reducing reagents, such as DTT or sodium cyanoborohydride, are required to stabilize the bond, there is no reduction of native disulfide bonds in proteins which can lead to protein denaturing and loss of activity.

No cross-linking

Unlike maleimido-succinimidyl based reagents, the use of HydraLinK™ reagents does not lead to cross-linking of proteins containing exposed cysteine residues, such as β-galactosidase and certain membrane proteins.

Compatible with solid phase synthesis

Reagents are available to incorporate aldehyde moieties on oligonucleotides during solid phase synthesis and hydrazine moieties on peptides during solid phase peptide synthesis.

Quantitation

The level of incorporation of both 2-hydrazinopyridyl and benzaldehyde can be easily quantified both spectrophotometrically and colorimetrically (Figure 4).

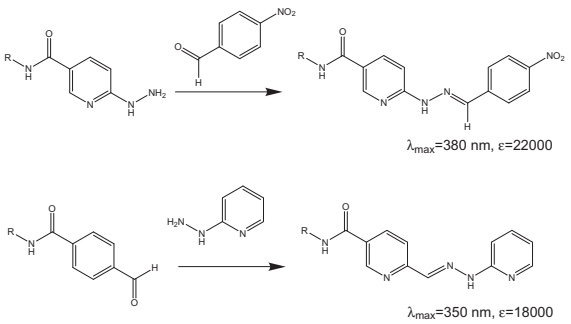


Figure 4: Reaction schemes for the quantification of 2-hydrazinopyridyl and benzaldehyde moieties incorporated on biomolecules.

Selective

The conjugation reaction is highly selective, eliminating non-specific interactions.

Versatile

The technology is ideal for use in micro-array formats for immobilization of peptides, proteins and DNA.

Applications

Protein/protein conjugation

Hydrazine and aldehyde amine modification reagents 1 (SANH) and 2 (SFB) modify proteins to incorporate hydrazine and benzaldehyde moieties, respectively, in a single step. The alkyl hydrazone protecting group on SANH readily hydrolyzes in acidic or neutral pH (4.5-7.4) and the liberated hydrazine moiety reacts with the aldehyde modified biomolecules to produce stable hydrazone conjugates (Figure 2). Unlike the classical maleimido/thiol approach, proteins modified with both hydrazines or aldehydes are stable for months. The degree of modification of incorporated reactive moieties (MSR: molar substitution ratio) can be readily determined by a colorimetric assay developed for these reagents.

SDS-PAGE data (Figure 5) show that hydrazino-modified BSA is readily coupled to aldehyde-modified IgG in a single step without the use of a reducing reagent and under mild conditions.

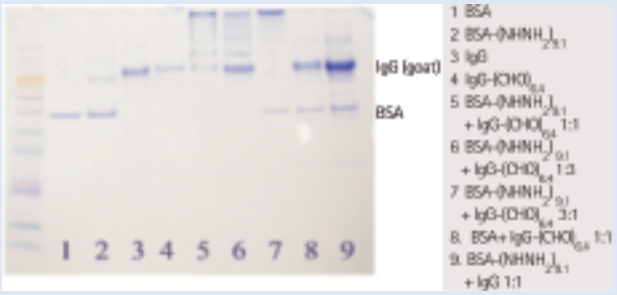


Figure 5: Demonstration of the conjugation of hydrazine modified BSA (lane 2) and aldehyde-modified IgG (lane 4). Reactions of 1/1, 1/3, 3/1 molar equivalents of BSA-$NHNH_2$ and IgG-CHO, lanes 5, 6 and 7 respectively. The subscript denotes the molar substitution ratio of modification groups on each protein. Lanes 8 and 9 are control reactions wherein unmodified BSA is reacted with IgG-CHO and BSA-$NHNH_2$ is reacted with unmodified IgG respectively.

Protein/oligonucleotide conjugates

The hydrazone/carbonyl bioconjugate couple has been developed to prepare oligonucleotide/protein conjugates as illustrated in Figure 6. In this experiment, IgG is reacted with 7.5 and 15 equivalents of SANH to incorporate hydrazine moieties on IgG. Following purification, the hydrazine-modified IgGs (IgG-$NHNH_2$) are reacted with 10 equivalents of 5'-aldehyde modified oligonucleotide in 0.1 M MES, 0.9% NaCl, pH 4.7 for 2 h. Examination of the gel indicates that there is 100% incorporation of oligonucleotide on the protein. Subsequently the proteins are transferred to a PVDF membrane and hybridized with a 5'-fluorescein-labelled oligonucleotide complementary to the first oligonucleotide. This experiment further demonstrates that the oligonucleotide remains available for hybridization. A 75mer 5'-aldehyde modified-oligonucleotide has been successfully conjugated to hydrazine-modified antibodies in excellent (60-75%) yield based on protein recovery.

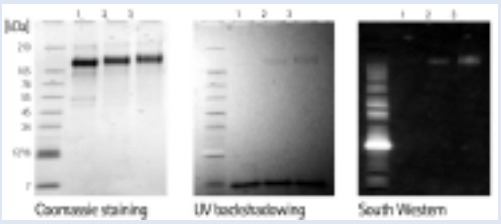
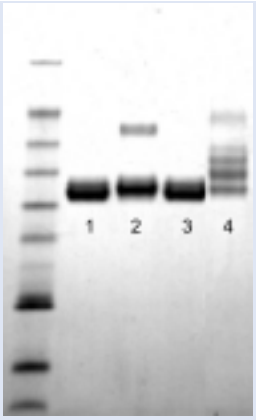


Figure 6: IgG (lane 1) is first modified to incorporate hydrazine moieties by reaction with SANH 7.5 equiv and 15 equiv. followed by the reaction of 5'—modified oligonucleotide (22 mer) lanes 2 and 3 respectively. The gel on the left was developed with Coomassie blue to show the protein and the center gel was visualized by UV-backshadowing demonstrating that the oligonucleotide is associated with the protein. The gel on the right is a south-western blot experiment wherein the protein is transferred to a PVDF membrane and the 5'-fluorescein-labelled oligonucleotide complementary to the covalently linked oligonucleotide hybridized to the oligo/protein conjugate. This further demonstrates that the biological activity of the oligonucleotide is retained following conjugation.

Glycoprotein/oligonucleotide conjugates

Hydrazine-groups have been directly incorporated on oligonucleotides using SANH (1) and the hydrazine-modified oligonucleotides can be directly conjugated to oxidized glycoproteins. Figure 7 shows the reaction product of 5'-hydrazine-modified oligonucleotide (prepared by the reaction of SANH with a 5'-amino-modified oligonucleotide) with periodate oxidized horseradish peroxidase. Simple incubation of the hydrazine-modified oligonucleotide with the protein produces the enzyme-oligonucleotide conjugate; no reduction reagents are required.



1. HRP
2. HPR-ox
3. HRP + H_2NNH-Oligo
4. HRP-ox + H_2NNH-Oligo

Figure 7: PAGE gel demonstrating the reaction of 5'-hydrazine-modified oligonucleotide (22 mer) with periodate oxidized horseradish peroxidase.(lane 4). Lanes 1, 2 and 3 are HRP, periodate-oxidized HRP and the control reaction of hydrazine-modified oligonucleotide with native HRP.

Oligonucleotide/peptide conjugates

The preparation of oligonucleotide/peptide conjugates using the hydrazine/aldehyde couple has also been demonstrated (Figure 8). In the following example, a 5'-aldehyde modified oligonucleotide (prepared as described above on solid support) was reacted with a 15mer peptide bearing an N-terminal acetone nicotinylhydrazone attached to an aminohexanoyl spacer. Simple addition of the hydrazine-modified peptide to the aldehyde-modified oligonucleotide (lane 1) directly yielded the peptide/oligonucleotide conjugate (lane 2) without the requirement of reducing reagents.

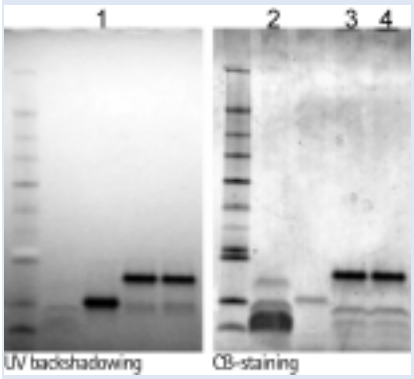


Figure 8: PAGE gel of the reaction of the N-terminus-hydrazone-modified peptide (2 equivalents, lane 2) with a 5'-aldehyde oligonucleotide (1 equivalent, lane 1); oligo-peptide conjugate, lanes 3, 4.

Protocols for use of HydraLinK™ Kit A to prepare Protein/Protein conjugates

The general scheme for the use of HydraLinK™ SANH and SFB is shown in Figure 9.

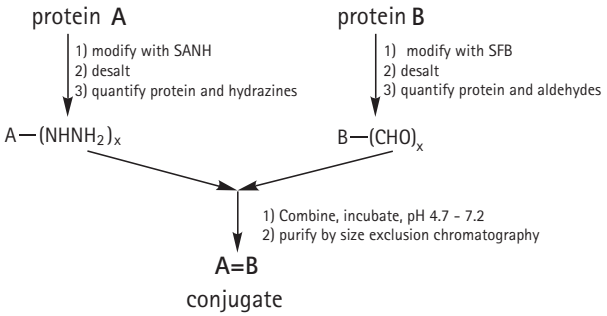


Figure 9: Preparation of a protein/protein conjugate using HydraLinK™ SANH and SFB.

Modification reactions

The resulting level of incorporation of the modification moiety, i.e. the molar substitution ratio (MSR), is a function of both the protein concentration and the number of equivalents of reagent added. As both the protein concentration and number of equivalents added are increased, so does the MSR increase. Over modification of a protein may result in precipitation of the modified protein and loss of biological activity. As the properties of proteins vary considerably, in some cases optimization of modification conditions will be required. As a rule of thumb, use 10 equivalents of reagent to protein, with a protein concentration of 4 mg/ml. This will usually yield 2.5-3 modifications /protein. The reaction should be carried out at pH 7.2-7.8. With the SHNH reagent, it is particularly important to measure the pH after the addition of this reagent, to ensure the reaction mixture does not become too acidic. The pH can be easily checked by spotting 0.5 - 1.0 µl of the reaction solution on a pH stick.
Before derivatization, proteins should be dialyzed into modification buffer and their concentration determined. It is recommended that the protein concentration be > 1.5 mg/ml. Amine containing buffers such as tris or glycine must be avoided. Following conjugation unreacted hydrazino or aldehyde functionalities can be capped by the addition of 2-sulfobenzaldehyde or 2-hydrazinopyridine. When conjugating peptides or DNA to proteins, it is only necessary to add the capping group complementary to the reactive group on the protein. For protein-protein conjugation, if one wishes to cap reactive groups on both proteins, the capping reagents should be added sequentially an hour apart to prevent cross-reaction. Twice the amount of the second capping reagent should be used to compensate for consumption by reaction with the first.

Method 1: SANH/SHNH modification

a. Modification

1) Prepare a solution of SANH or SHNH (3.0 mg) in 2 ml DMF (5mM).
2) Add 10 eq. of SANH/DMF solution to a 50 µM protein solution in modification buffer such that the percentage of DMF in the reaction mixture is 5-10% of the volume. Vortex the mixture to homogeneity and check pH. Adjust with 1 M $NaHPO_4$ to 7.4, if necessary.
3) Incubate at room temperature for a minimum of 2-3 hours
4) Desalt using a gel filtration matrix such as Sephadex G-25, dialysis or diafiltration. Combine protein containing fractions, reduce volume, and determine protein concentration using a colorimetric method such as a BCA or Bradford assay.

b. Quantitation of hydrazine incorporation

1) Prepare a buffered solution of 0.5 mM p-nitrobenzaldehyde by dissolving p-nitrobenzaldehyde (50 µmol, 7.6 mg) in an organic solvent (1 ml), such as MeOH, DMF or DMSO, and adding 10µl to 990 µl of an acidic buffer, preferably 100 mM acetate, pH 5.0 or 100 mM MES, pH 5.0, to give a solution of desired concentration.
2) Add an aliquot of the hydrazine-modified protein solution (containing 10 µg) to the p-nitrobenzaldehyde solution (100 µl) and incubate at 37°C for 1 hour or at room temperature for 2 h. Calculate the protein concentration of this solution. Determine the A_{380} of the solution against a blank prepared by addition of an equal aliquot of buffer in reagent solution.
3) The hydrazine/protein MSR is calculated using the following equation.
$MSR=(Abs_{sample}-Abs_{blank})/(\text{Protein conc. of test solution} \times 22000 \times \text{path length of cuvette})$

Method 2: SFB modification

a. Modification

1) Prepare a 5 mM solution of SFB (2.5 mg) in 2 ml DMF
2) Add 10 eq. of SFB/DMF solution to a 50 µM protein solution in modification buffer such that the percentage of DMF in the reaction mixture is 5 -10% of the volume.
3) Incubate at room temperature for a minimum of 2-3 hours.
4) Desalt using a gel filtration matrix such as Sephadex G-25, dialysis or diafiltration.
5) Combine protein containing fractions, reduce volume, and determine protein concentration using a colorimetric method such as a BCA or Bradford assay.

b) Quantitation of aldehyde incorporation

1) Prepare a 0.5 mM buffered solution of 2-hydrazinopyridine · 2HCl by dissolving 2-hydrazinopyridine · 2HCl (50 µmol, 9.1 mg) in a water (1 ml) or buffer and adding 10 µl to an acidic buffer (990 µl), such as 100 mM acetate, pH 5.0 or 100 mM MES, pH 5.0.
2) Add an aliquot of the benzaldehyde-modified protein solution (containing 10 µg) to the 2-hydrazinopyridine · 2HCl solution (100 µl) and incubate at 37°C for 1 hour or at room temperature for 2 h. Calculate protein concentration of this solution. Determine the A_{350} of the solution against a blank prepared by addition of an equal aliquot of buffer in reagent solution.
3) The hydrazine/protein MSR is calculated using the following equation:
$MSR=(Abs_{sample}-Abs_{blank})/(\text{Protein conc. of test solution} \times 18000 \times \text{path length of cuvette})$

Conjugation reaction

The successful conjugation of two modified proteins is dependent on protein concentration, protein modification, pH, reaction time, buffer and pH. Hydrazone formation is an acid catalyzed reaction and the optimal pH is 4.7-5.0 but the reaction also occurs up to pH 7.2, albeit with slower kinetics. As HydraLink™ modification groups have extended stability the concentration of the starting proteins in the conjugation reaction can be as low as 0.1 mg/ml. The conjugation reaction can also be performed at 37°C if required.

The extent of crosslinking of two proteins is a function of the MSR (number of reactive groups/protein), i.e. if one or both proteins have > 5 functional moieties/protein a conjugate of very high molecular weight will be produced.

Reagents/buffers required

HydraLink™ Kit A

Modification buffer: 100 mM phosphate, 150 mM sodium chloride, pH 7.2-7.4 Note: PBS (10 mM phosphate, 150 mM sodium chloride, pH 7.2) is not recommended for protein modifications due to poor buffering of this low phosphate buffer.

Conjugation buffer: The optimal pH for the formation of a hydrazone is 4.7; however, buffers of higher pH can also be used, but this will lead to an increase in reaction times. The following buffer have been successfully used to produce bioconjugates:

pH 4.7: 100 mM MES, 150 mM NaCl
pH 5.0: 100 mM acetate
pH 6.0: 100 mM citrate, 150 mM NaCl
pH 7.2: 100 mM phosphate, 150 mM NaCl

Note: For antibody conjugations citrate buffer, pH 6.0 is recommended to maintain immunoreactivity of the antibody.

Desalting columns or diafiltration apparatus: for isolation modified protein from excess reagent.

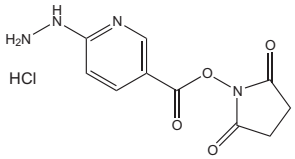
Method 3: Conjugation

1) Mix solutions of SFB and SANH-modified proteins in desired molar ratios in conjugation buffer and incubate overnight. The extent of reaction can be determined by performing a PAGE gel analysis on an aliquot of the reaction mixture. Unreacted hydrazine or aldehyde groups on the target protein can be capped by the addition of 2-sulfobenzaldehyde or 2-hydrazinopyridine.
2) Isolate of protein/protein conjugate by standard gel filtration or ion exchange chromatographic methods.

Product No.	Product	Quantity

Ordering Information

Reagents for conjugation



01-63-0118 **HydraLinK™ SHNH Reagent#** — 25 mg, 100 mg

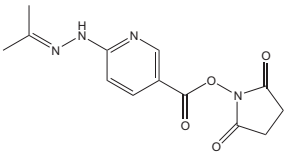
NEW

Succinimidyl 2-hydrazinonicotinate hydrochloride

$C_{10}H_{10}N_4O_4$; M.W.: 286.7

⚠ Prolonged storage: ≤ -18°C; keep cool and dry. Store under argon.

Reagent to modify amine-containing biomolecules or surfaces to directly incorporate 2-hydrazinopyridine moieties.



01-63-0117 **HydraLinK™ SANH Reagent#** — 25 mg, 100 mg

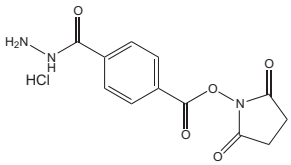
NEW

Acetone 5-(Succinimidyloxycarbonyl)-pyridine-2-ylhydrazone

$C_{13}H_{14}N_4O_4$; M.W.: 290.2

⚠ Prolonged storage: ≤ -18°C; keep cool and dry. Store under argon.

Reagent to modify amine-containing biomolecules or surfaces to directly incorporate 2-hydrazinopyridine moieties.



01-63-0119 **HydraLinK™ SHTH Reagent#** — 25 mg, 100 mg

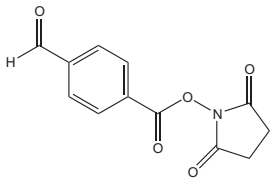
NEW

5-(Succinimidyloxycarbonyl)-benzoylhydrazinium chloride

$C_{12}H_{11}N_3O_5$; M.W.: 313.7

⚠ Prolonged storage: ≤ -18°C; keep cool and dry. Store under argon.

Reagent to modify amine-containing biomolecules or surfaces to directly incorporate benzoylhydrazine moieties.



01-63-0120 **HydraLinK™ SFB Reagent#** — 100 mg

NEW

Succinimidyl 4-formylbenzoate

$C_{12}H_9NO_5$; M.W.: 247.1

⚠ Prolonged storage: ≤ -18°C; keep cool and dry.

Reagent to modify amine-containing biomolecules or surfaces to directly incorporate aldehyde moieties.

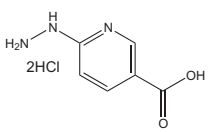
08-01-0001 **HydraLinK™ Kit A#** — 1

NEW

HydraLinK™ SANH Reagent (25 mg)
HydraLinK™ SFB Reagent (100 mg)
HydraLinK p-Nitrobenzaldehyde Reagent (100 mg)
HydraLinK™ 2-Hydrazinopyridine Reagent (100 mg)
HydraLinK™ 2-Sulfobenzaldehyde Reagent (50 mg)

⚠ Prolonged storage: ≤ -18°C; keep cool and dry. Store under argon.

Complete kit for modification of proteins with hydrazines and aldehydes and their quantification.



01-63-0123 **HydraLinK™ 6-HNA Reagent#** — 100 mg, 500 mg

NEW

6-Hydrazinonicotinic acid

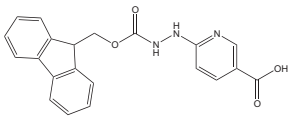
$C_6H_7N_3O_2$; M.W.: 153.1

⚠ Prolonged storage: Keep cool and dry. Store under argon.

Reagent to modify amine-containing biomolecules or surfaces to directly incorporate 2-hydrazinopyridine moieties.

Product No.	Product	Quantity

Reagents for solid phase synthesis



01-63-0121 **HydraLinK™ 6-Fmoc-HNA Reagent#** — 100 mg, 500 mg

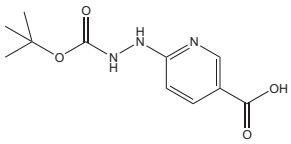
NEW

Fmoc-6-hydrazinonicotinic acid

$C_{21}H_{17}N_3O_4$; M.W.: 375.3

⚠ Prolonged storage: ≤ -18°C; keep cool and dry.

Reagent for the introduction of the 2-hydrazinopyridine group during Fmoc SPPS. 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). This product is protected by US Patents 5,206,370, 5,420,285, 5,753,520, and 5,769,778, and EU Patent 0.384,769.



01-63-0122 **HydraLinK™ 6-Boc-HNA Reagent#** — 100 mg, 500 mg

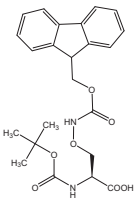
NEW

Boc-6-hydrazinonicotinic acid

$C_{11}H_{15}N_3O_4$; M.W.: 253.3

⚠ Prolonged storage: ≤ -18°C; keep cool and dry.

Reagent for the introduction of the 2-hydrazinopyridine group during Fmoc SPPS. 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). This product is protected by US Patents 5,206,370, 5,420,285, 5,753,520, and 5,769,778, and EU Patent 0.384,769.



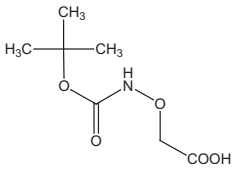
04-12-0242 **Boc-Ams(Fmoc)-OH** — 1 g

N-α-t-Boc-O-(Fmoc-amino)-L-serine

$C_{23}H_{26}N_2O_7$; M.W.: 442.5

This novel building block is a useful tool for the synthesis of large, branched and cyclic peptides by chemoselective ligation.

It can be introduced using standard coupling methods. Following cleavage and side-chain deprotection, peptides are produced bearing a pendant hydroxylamine moiety. At pH 3-5 in aqueous media, these peptides ligate with peptides, or other entities, possessing an aldehyde functionality. The reaction is extremely selective and is compatible with all standard amino-acid residues, with the exception of N-terminal cysteine.



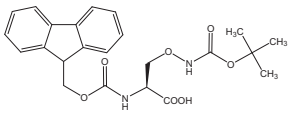
01-63-0060 **Boc-amino-oxyacetic acid** — 1 g, 5 g

Boc-NH-O-CH_2COOH

$C_7H_{13}NO_5$; M.W.: 191.2

TLC: $CHCl_3$:MeOH:AcOH 32 % (15:4:1), purity: ≥ 98.00%.

This reagent can be used to introduce a hydroxylamine functionality to N-terminal or side-chain amino groups. Hydroxylamine-labeled peptides prepared in this manner can be ligated in aqueous solution at pH 3.5 to aldehyde-containing peptides via oxime formation.



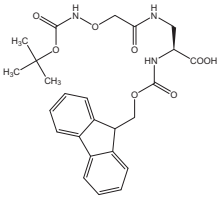
04-12-1209 **Fmoc-Ams(Boc)-OH** 1 g

N-α-Fmoc-O-(t-Boc-amino)-L-serine

$C_{23}H_{26}N_2O_7$; M.W.: 442.5

This novel building block is a useful tool for the synthesis of large, branched and cyclic peptides by chemoselective ligation.

It can be introduced using standard coupling methods. Following cleavage and side-chain deprotection, peptides are produced bearing a pendant hydroxylamine moiety. At pH 3-5 in aqueous media, these peptides ligate with peptides, or other entities, possessing an aldehyde functionality. The reaction is extremely selective and is compatible with all standard amino-acid residues, with the exception of N-terminal cysteine.



04-12-1185 **Fmoc-Dpr(Boc-Aoa)-OH** 1 g / 5 g / 25 g

N-α-Fmoc-N-β-(N-t.-Boc-amino-oxyacetyl)-L-diaminopropionic acid

$C_{25}H_{29}N_3O_8$; M.W.: 499.5

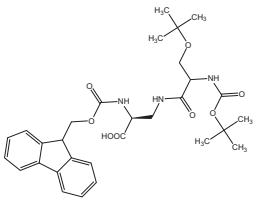
TLC: $CHCl_3$:MeOH:AcOH 32 % (15:4:1), purity: ≥ 98.00%.

$CHCl_3$:MeOH:AcOH (77.5:15:7.5), purity: ≥ 98.00%.

HPLC: purity: ≥ 95.00%.

An excellent derivative for the chemoselective ligation of unprotected peptides in aqueous media [1] via oxime formation. This derivative introduces a hydroxylamine functionality which can couple with aldehyde groups present in another peptide unit.

[1] F. Wahl & M. Mutter (1996) *Tetrahedron Lett.*, **37**, 6861.



04-12-1186 **Fmoc-Dpr(Boc-Ser(tBu))-OH** 1 g / 5 g / 25 g

N-α-Fmoc-N-β-(O-t.-butyl-N-t.-Boc-L-serinyl)-L-diaminopropionic acid

$C_{30}H_{39}N_3O_8$; M.W.: 569.6

TLC: $CHCl_3$:MeOH:AcOH 32 % (15:4:1), purity: ≥ 98.00%.

HPLC: purity: ≥ 95.00%.

An excellent derivative for the chemoselective ligation of unprotected peptides in aqueous media [1]. Following TFA cleavage, treatment with sodium periodate converts the pendant serine moiety to glyoxylic acid, which can couple via an oxime bond with another peptide unit containing an hydroxylamine functionality introduced using Fmoc-Dpr(Boc-Aoa)-OH (04-12-1185) or Boc-aminooxyacetic acid (01-63-0060), or can couple to peptide containing a N-terminal Cys residue via thiazolidine formation.

[1] F. Wahl & M. Mutter (1996) *Tetrahedron Lett.*, **37**, 6861.

Merck Biosciences AG · Switzerland
Weidenmattweg 4
4448 Läufelfingen
Phone +41 (62) 285 2525
Fax +41 (62) 285 2520

www.novabiochem.com

NVT205-NP