FT-42252A

NHS ASA, SASD

Photoreactive & Primary amine reactive crosslinkers

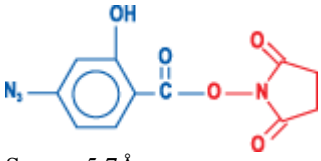
Products descriptions

Catalog number: UP42252B , 100mg UP42252A, 50mg

Name: **NHS-ASA**

Formula : N-Hydroxysuccinimidyl-4-azidosalicylic acid
M.W.= 276.2, CAS[96602-46-9]

Storage +4°C; (K)



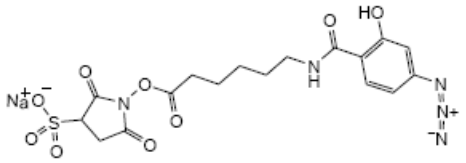
Spacer 5.7Å

Catalog number: 22372A , 50mg

Name: **SulfoNHS-lc-ASA**

Formula : sulfosuccinimidyl[4-azidosalicylamido]hexanoate
M.W.= 491, Spacer 18.0Å

Storage -20°C; (M)

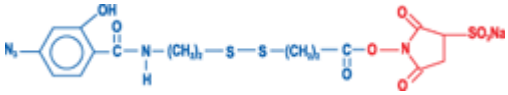


Catalog number: UP40901B , 100mg

Name: **SASD**

Formula : sulfosuccinimidyl[4-azidosalicylamido]hexanoate
M.W.= , Spacer Å; (M)

Storage -20°C; (M)



a unique sulfo-NHS-ester, photoactivatable phenyl azide that is both iodinatable & cleavable

Storage : +4°C, protect from moisture and light (L)
-20°C (sulfonated derivatives) , protect from moisture and light (M)

General Considerations

NHS-ASA and its derivatives are heterobifunctional crosslinkers which have:
-a reactivity toward amines through its N-hydroxysuccinimide amino reactive group
-an other induced under UV exposure, that is non selective, though its a hydroxyphenyl azide group
-a spacer (aryl type) – cleavable for SASD -

The NHS group is usually reacted first with a protein or molecule because this group is more labile. After desalting from by-products, NHS-activated molecule bear the arylazide groups that can couple by photoactivation a second molecule.

Hydroxy-substituted NHS-ester arylazide cross-linkers are useful for subunit and near-neighbor studies. The non-cleavable cross-linkers are useful in applications requiring reducing conditions in subsequent steps while maintaining their cross-links. The cleavable crosslinkers are useful for transferring radioactive iodine from one molecule to its associated molecule.

Photoreactive cross-linkers can be iodinated on the phenyl ring. As the radioactive label is not removed after cleavage by a reducing reagent, labeled protein can be identified by autoradiography.

Scientific and Technical Information

- **Solubility**:
  SulfoNHS-ASA can be dissolved directly in aqueous buffers, but should be used immediately. High concentrations (mM) may however require the use of organic solvant.
  NHS-ASA should be solubilized in DMSO or DMF prior dissolution in aqueous buffers, usually by 1:10to100 dilution depending on desired concentration. It may not keep in solution above 5 final mM.
  Buffers should not contain amines (i.e. Tris, Glycine). Unused reagents should be discarded, as aqueous solutions will hydrolyze rapidly. Storage of unused organic solution and powder is not recommended, requiring controlled conditions.

- The chemical group **N-hydroxysuccinimydyl (NHS)** reacts in aqueous phase on primary ($-NH_2$) and secondary amines (=NH) (in fact on its deprotonated form), optimally at neutral pH or higher (7-9): amines present in proteins (Lys aminoacid) and in a lower proportion on NH_2 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 derivatized. The reaction results in the release of N-hydroxysuccinimide.
  Suitable conjugation buffers include phosphate, borate, carbonate, and HEPES, and typical buffer concentrations range from 50-200 mM. The pH of the NHS reaction should be between pH 7 and 9. Buffers should not contain primary amines or sulfhydryls.
  Usual concentrations are 2-50 fold molar excess of NHS cross-linker over protein, but this also depends on the concentration of the molecule.

- The **sulfonyl moeity** ($NaSO_3$) introduces a hydrophilic group, that allows the product not to cross biological membranes. This is particularly useful to modify, in situ on cells, proteins presented outside membranes, and if one wants to avoid the modification of intracellular proteins that may affect further analysis. An other interest of the sulfonyl group is to permit the solubilization of the product directly in aqueous buffers, up to 10mM, avoiding the use of organic solvents like DMSO or DMF, that are possibly deleterious to cells or applications.

- The **spacer arm** of NHS-ASA is short (5.7 angstroms), longer (18A) for NHS-lc-ASA, or long and cleavable for SASD. See SANPAH and SAND for longer spacers, or PEG reagents.

- The **nitrophenyl azide** group, upon UV light exposure, forms a reactive nitrene group. Photolysis occurs at 300nm-370nm (optimal at 320nm), a condition that limits damage to biomolecules by irradiation. The nitrene group yields various reactions*: addition to double bonds, insertion into C-H and N-H sites, or subsequent ring expansion to react with a nucleophile (favored reaction in presence of primary amines) (Buchmueller 2003, 036). Many other reactions have been described in presence of catalysts: Photocatalytic reduction of aromatic azides to amines using CdS (Warrier 2004).
  *: See also NHS-Diazirine crosslinkers for similar reactions but with a more reactive group than the nitrophenyl azide.

•*Note[c]* •Use a **UV lamp** that irradiates between 320-370 nm, i.e. with 365nm bulb. High-wattage lamps are more effective and require shorter exposure times, while lower-wattage lamps(i.e. a hand-held 6 watt lamp) will result in lower crosslinking efficiency. +
Filters that remove light at wavelengths below 300 nm are necessary for mercury vapor lamps. Do not use UV lamps that emit light at 254 nm as this wavelength causes proteins and DNA to photo-destruct.
•Samples **irradiation conditions** should be optimized. As guideline, for efficient UV irradiation,
-place the lamp above a shallow, uncovered reaction vessel, rotate the sample.
-the distance from lamp to samples may be 20cm for > 150watts lamps a 300 nm filter, or 3-5cm for a 15watt lamp, or 1cm for a 8watts lamp without filter.
-total UV irradiation time should be less than 15 minutes for crosslinking of live cells.

Guide lines for use:

1) iodinate the crosslinker (optional)

2) react the NHS-ester moiety to primary amines of molecule one

3) desalt to remove excess non-reacted crosslinker

4) react the activated molecule 1 to desired molecule2 : mix and irradiate by UV light (320-350nm)

Directions for Use

Note: Perform all steps in darkroom conditions until after the photoactivation of the cross-linker.

Iodination of Cross-linkers [r]

Note: This method uses IODO-GEN® immobilized oxidant which must be prepared ahead of time. Do not use chloramine-T as this requires a reducing agent which will prematurely cleave the SASD.
Note: Perform the iodination as quickly as possible to limit the hydrolysis of the NHS ester.

1. Prepare 0.5 mM Working Solutions

NHS-ASA

a. Dissolve about 1.5 mg NHS-ASA in 100 µl of DMSO in a 100 µl small reaction vial.

b. Make a 1:200 dilution of the NHS-ASA solution by adding 199 µl of 0.1 M PBS pH 7.4, to 1.0 µl of NHS-ASA Solution. Mix well. This makes 200 µl of a 0.5 mM working solution.

c. If NHS-ASA solubility is a problem at this point, more DMSO can be added to the solution.

SASD and Sulfo NHS-LC-ASA

Prepare 200µl of a 0.5mM working solution by dissolving the sulfonated cross-linker in 0.1M PBS, pH 7.4.

2. Radiolabel the Cross-linker

a. Quickly add the working solution into the vial treated with IODO-GEN® Iodination Reagent. Add 40 µCi Na-$_{125}$I in 10 µl 0.1 M PBS pH 7.4.
b. Allow the reaction to proceed for exactly 30 seconds, then proceed to Step 3.

Molecular Cross-linking (ex. cross-linking IgG to Gelonin) [r]

3. Conjugation of Radiolabeled Cross-linker to IgG (MW 150,000) using SASD

a. Immediately pipet the iodinated cross-linker into a tube containing 2.0 mg of human IgG (13nmol) in 300 µl of PBS, pH 7.4.

b. React for 1 hour at room temperature.

c. Remove unreacted SASD by applying the reaction mixture to a 5 ml Desalting Column that has been equilibrated with 10 column volumes of PBS, pH 7.4. Elute fractions with PBS and collect 500 µl fractions. The derivatized IgG should elute in the void volume. For a quick check, use a gamma counter and use the first eluting radioactive 500 µl fraction for the next step. Properly dispose of the used columns, along with the other radioactive waste, as this will contain the unincorporated 125I.

4. Conjugation of IgG to Gelonin (MW 30 000)

a. Add 1 mg of gelonin dissolved in 500 µl PBS to the first radioactive fraction from Step 3c.

b. Incubate the reaction mixture for 15 minutes at 37°C. Irradiate with long-wave UV light for 10 minutes at room temperature, or flash with bright light for 1-3 seconds (5 camera flashes work well).

c. After the reaction, leftover unconjugated reactants can be removed by any common size exclusion method such as dialysis or spin concentration. Be sure to properly dispose of any unreacted radioactive materials.

5. Cleavage of SASD

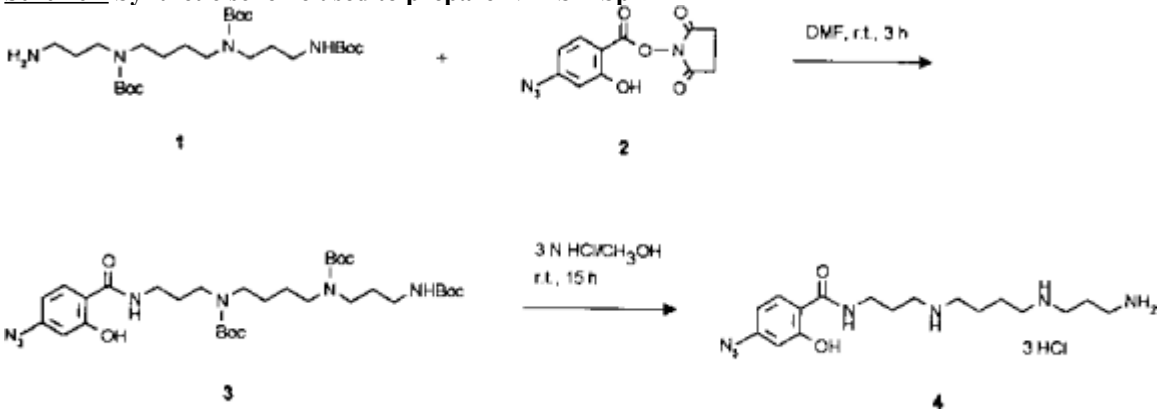
a. SASD can be cleaved by incubation in 100 mM mercaptoethanol for 1 hour at 37°C.

b. The mercaptoethanol can be removed by dialysis, desalting column, or spin concentrator and the resulting cleaved molecules can be stored in a buffer containing 10 mM EDTA to preserve the sulfhydryls for a limited time.

Synthesis of photoprobes[(Donna 1997)]

The synthetic route used to prepare the photoprobe precursor **4** (N^1-ASA-Spm) is illustrated in Scheme 1. All chemical reactions were performed under an atmosphere of dry N_2 and low light conditions. Compounds **1** and **2** have been described previously (**1**[Jasys 1990] **2**[Dupuis 1987]). Reaction of the t-butyloxycarbonyl (Boc)-protected spermine derivative **1** (tri-Boc-Spm) with the photoreactive donor **2** [*N*-hydroxysuccinimidyl-4-azidosalicylic acid (NHS-ASA)] yielded the intermediate **3** (N^1-ASA-tri-Boc-Spm). Removal of the N-Boc-protecting groups from compound **3** with 3 M HCl/methanol afforded the target compound **4** as the trihydrochloride salt.

Scheme 1 **Synthetic scheme used to prepare N^1-ASA-Spm**



References – NHS-ASA

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Other information

For R&D *in vitro* use only, not for diagnostics, food or drug uses.

Related products and documents:

See BioSciences Innovations catalogue and e-search tool.

*Other crosslinkers:

- SANPAH #09649A (non iodinatable photoreactive and amine reactive crosslinkers)
- Diazirine chemistry (photoreactive): SDA reagents #DW8531 (better photostability in normal light than phenyl azide groups, hence more efficiently activated by long-wave UV light)
- MAL-PEOx-NHS #AL6580 (hydrophilic spacer), SMCC #17412A, GMBS #UP49608A & Sulfo-GMBS #, MBS #UP21608A, SMPB #UP28072A.
- Hydralink Chemistry: Conjugation kit #BL1501 and crosslinkers (SANH #BL9270, MHPH #BL9401)

*Associated product

- Phosphate-buffered saline (PBS) #UP687213A, TBS #UP09154D.
- Quenching Buffer (1 M Tris•HCl, pH 8.0) #
- Desalting Columns # and Dialysis devices or tubings #BB101a (i.e.Cellusep dialysis tubings)
- Protein Assays: Coo Assay #UPF86400, BC Assay UP40840A

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