Application Note – N-*o*-Ns protection

Introduction

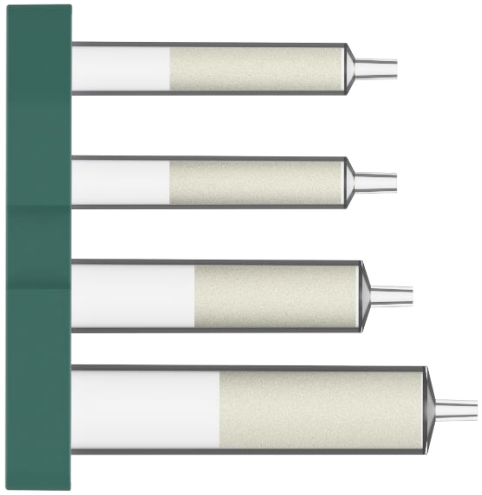
Nitrogen, as a key element with existence in diverse forms, participates in various metabolic and physiological functions in the living organisms. As the most important N-centered functional group, amino group presents widely in small molecules, peptides and proteins. Due to its nucleophilic nature, amino group often requires protection in the multistep synthesis as a means to block reactive sites with close proximity and sometimes induce more favorable solubility for reaction handling and product purification. Many protecting groups for amino group have been developed in the past century and they have become a set of must-have tools for all phases in research and development. Carbamates are an important class of protecting groups, such as *tert*-butyl carbamate (Boc), benzyl carbamate (Cbz) and 9-fluorenylmethyl carbamate (Fmoc). As an alternative to carbamates, it was established that using nitrobenzenesulfonamides (Ns) is an efficient and versatile synthetic method for the protection of amines. This class of protecting group represent an interesting class because of, 1) its stability against many acidic, basic conditions; 2) both installation (with Ns-Cl) and removal (under mild conditions) are relatively straightforward; 3) its role as an activating group for the synthesis of secondary amines from primary amines.

Though *o*-Ns-Cl (2-Nitrobenzenesulfonyl chloride) is easily used for N-*o*-Ns protection, it is a corrosive reagent, which may cause severe skin burns and eye damage. Usually N-*o*-Ns protection reaction requires a basic aqueous workup to remove the excess amount of *o*-Ns-Cl, which is tedious and less ideal. By using pre-packed *o*-Ns-Cl cartridge and solid-phase purification method, *o*-Ns protection has become safer and more user-friendly.

Using the approach in this application note, the Synple Chem synthesizer offers an easy and fast automated method for the *o*-Ns protection of primary and secondary free amines. Amine salts are not supported by this method and have to be free-based before.

Cartridge Contents

The cartridge contains a set of reagents to carry out an *o*-Ns protection reaction at a scale up to 0.8 mmol.



–

–

solid-supported triethylamine / *o*-Ns-Cl

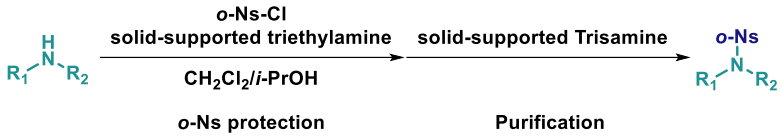
solid-supported Trisamine

This method can be used for the following transformations:

- *o*-Ns protection of alkyl amines.

Reaction Scheme

This section describes the general course of the *o*-Ns protection:



Reaction Procedure

1) Solubilization of amine

The starting amine in the vial is dissolved in CH_2Cl_2/*i*-PrOH (1:1, 4 mL).

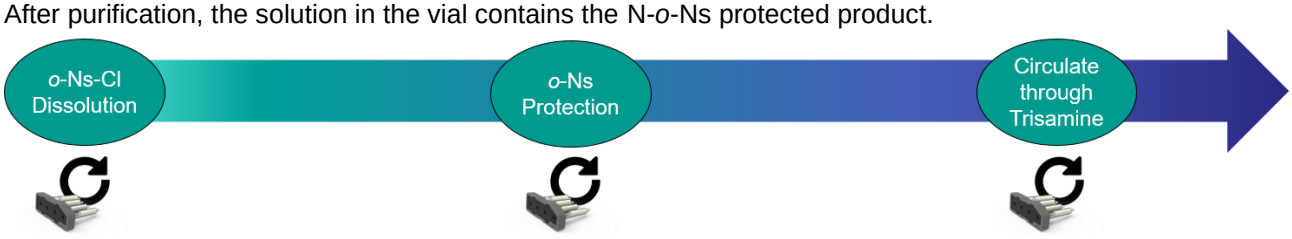
2) N-*o*-Ns formation

In the first step, the pre-mixed solution of amine is circulated through compartment 3 (*o*-Ns-Cl and solid-supported triethylamine) at 1 mL/min at room temperature for 3 hours. Compartment 3 is further rinsed with MeCN, which goes into the vial.

3) Purification

The reaction mixture is then circulated through compartment 4 (solid-supported Trisamine) at 2 mL/min for 1.5 hour. Compartment 4 is further rinsed with MeCN, which goes into the vial.

After purification, the solution in the vial contains the N-*o*-Ns protected product.



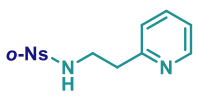
Substrate Scope

Tolerated functional groups

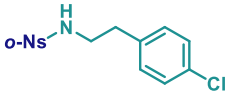
A wide range of functional groups are tolerated including alcohols and thiols, alkenes, amides, aryl halides, carbamates, esters, ethers, ketals, nitro groups, silyls and various heterocycles (imidazole, pyridine, pyrimidine, quinoline, thiophene, etc.),

Example substrate scope (from 0.8 mmol free amine)

Primary amines

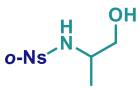


99%

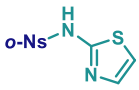


98%

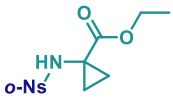
Traces



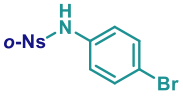
92%



Traces

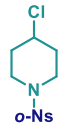


83%

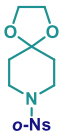


20%

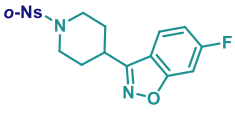
Secondary amines



99%

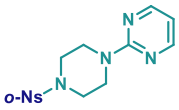


98%

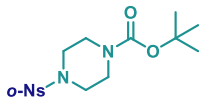


84%

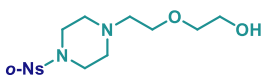
Traces



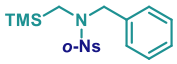
99%
99% (0.2 mmol)



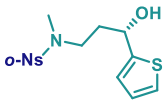
99%



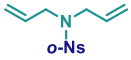
98%



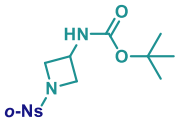
96%



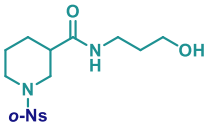
99%



98%



99%

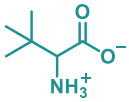


98%

Identified Chemistry Limitation

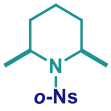
Insoluble starting materials

Zwitterions like amino acids or compounds containing an amine and a carboxylic acid are poorly soluble in CH_2Cl_2/*i*-PrOH (1:1).



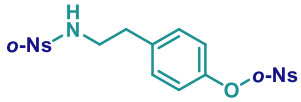
Reactivity

Sterically hindered secondary amines reacted more slowly in the *o*-Ns protection, therefore longer reaction time is required (see Substrate Scope, secondary amines). In these cases, unreacted amines were observed in the final solution of the product.



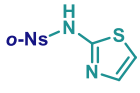
Traces

Amines containing phenols led to the double protection of the amine and the oxygen from the phenol.

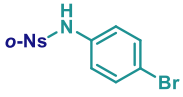


56%

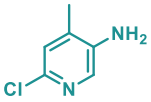
Additional list of identified compounds with low to no reactivity or insolubility issues:



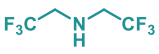
Traces



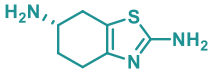
20%



0%



0%



Insoluble

Reaction Parameter Editing

Editing parameters:

Parameter 1	Reaction time of *o*-Ns protection step (seconds) e.g. 12 hours = 43200 seconds

Enabling and Disabling parts:

Part 1: Purification step:
The purification step of the sequence can be disabled. Then synthesizer will then provide the crude product in solution in the vial after the protection step.

Reaction Planning

Solubility of reactants
The starting amine shall be soluble in a solution of CH_2Cl_2/*i*-PrOH (1:1). Sonication and heating up the vial up to 35°C may help for the solubilisation of the amine.

Tolerance of air and/or moisture
N-*o*-Ns protection reaction using Synple Chem synthesizer is insensitive toward air and moisture.

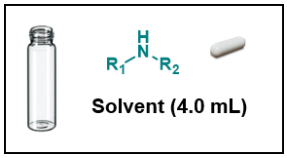
Sample Preparation

Precaution
To ensure a successful reaction in the Synple Chem synthesizer, automated CH_2Cl_2 wash shall be run before setting up an *o*-Ns protection reaction.

Setup

Components for sample preparation:

- Vial
- Amine (up to 0.8 mmol)
- Stir bar
- Reaction solvent (4 mL - CH_2Cl_2/*i*-PrOH = 1:1)



Guide of solvents and ratios for sample preparation:

1) **Alkyl amines (0.8 mmol)**
   Anhydrous CH_2Cl_2 (2.0 mL, 99.8%, amylene stabilized) and *i*-PrOH (2.0 mL, 99.8 %).
2) **Tips for sample preparation**
   Sonication may help dissolving poorly soluble materials.
   Heating-up the vial up to 35°C may help dissolving poorly soluble materials.
   Pre-grinding the solid alkyl amines may help dissolving poorly soluble materials.

Machine Solvents for the use with *o*-Ns protection cartridge

Please connect the following solvent to the color-coded solvent lines:

	S1: CH_2Cl_2, 99.8% anhydrous, 50 ppm amylene tolerate
	S2: MeCN, >99.9%
	S3: MeOH, >99.9%
	S4: –
	S5: –

Machine Cleaning after N-*o*-Ns Protection Reaction

1) Run automated MeOH wash after the *o*-Ns protection reaction.
2) Run automated CH_2Cl_2 wash before starting a new *o*-Ns protection reaction.

Solvent Consumption and Run Time

SEQUENCE RUNTIME	
Reaction Sequence	**Time**
Nosyl protection	4 h 44 min

SOLVENT COMSUMPTION FOR BOC DEPROTECTION	
For Reaction Setup	**Amount**
Dichloromethane (CH_2Cl_2)	2 mL-
iso-Propanol (*i*-PrOH)	2 mL
Machine Solvents	
Dichloromethane (CH_2Cl_2)	20 mL
Acetonitrile (ACN)	31 mL