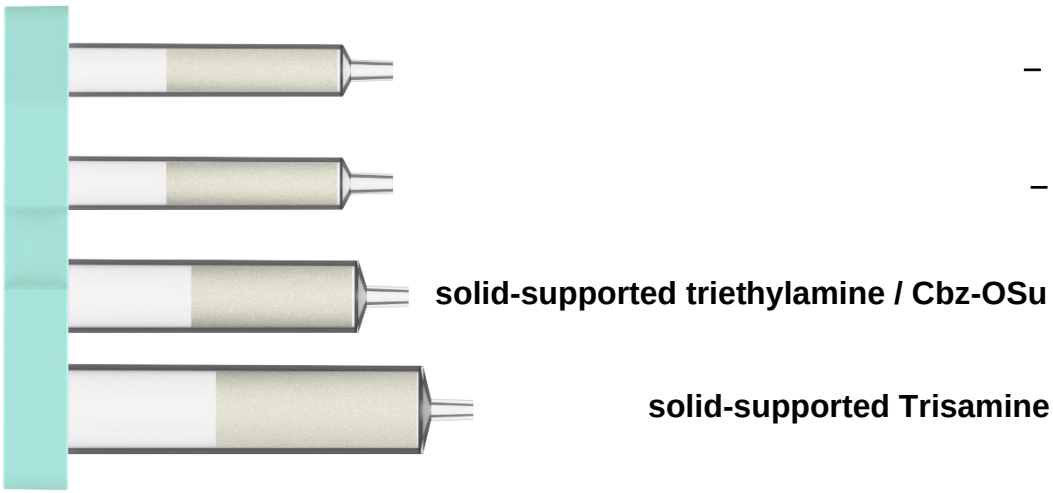
Application Note – N-Cbz protection

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). Among them, Cbz is one of the most broadly used for, 1) its stability against many acidic, basic or oxidative conditions and inert toward many electrophilic reagents; 2) both installation (with Cbz-Cl) and removal (under reductive conditions) are relatively straightforward.

Though Cbz-Cl (benzyl chloroformate) is easily used for N-Cbz protection, it is a highly toxic reagent which may induce cancer. Cbz-Cl is also a lachrymator with an acrid odor, therefore its handling may pose certain safety issue in the laboratory. Usually N-Cbz protection reaction requires a basic aqueous workup to remove the excess amount of Cbz-Cl, which is tedious and less ideal. To avoid the usage of Cbz-Cl, Cbz-OSu (N-(benzyloxycarbonyloxy)succinimide) represents as a non-toxic alternative. By using pre-packed Cbz-OSu cartridge and solid-phase purification method, N-CBz 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 N-Cbz protection of primary and secondary amines and amine salts.



Cartridge Contents

The cartridge contains a set of reagents to carry out a N-Cbz protection reaction at a scale up to 0.8 mmol.

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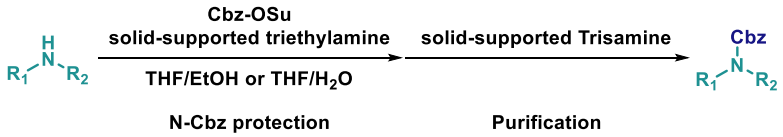
Cartridge product numbers: B031, B131

This method can be used for the following transformations:

- Cbz protection of alkyl amines.
- Cbz protection of alkyl amine salts.

Reaction Scheme

This section describes the general course of the N-Cbz protection:



Reaction Procedure

1) Solubilization of amine or amine salt

The starting amine in the vial is dissolved in THF/EtOH (3:1), while the starting amine salt is dissolved in THF/H_2O (3:1).

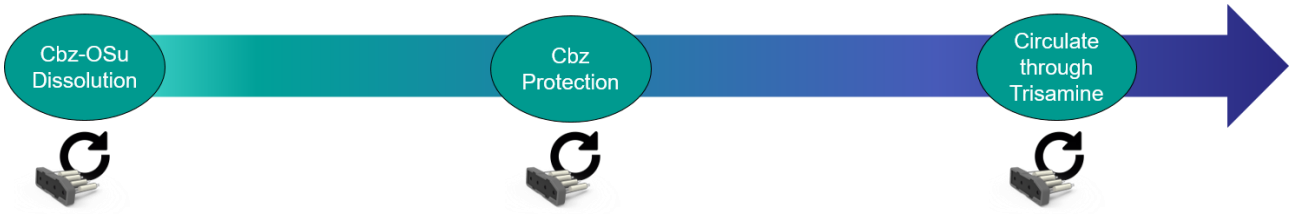
2) N-Cbz formation

In the first step, the pre-mixed solution of amine or amine salt is circulated through compartment 3 (Cbz-OSu and silica-supported triethylamine) at 1 mL/min at room temperature for 3 hours. Compartment 3 is further rinsed with CH_2Cl_2, which goes into the vial.

3) Purification

The reaction mixture is then circulated through compartment 4 (silica-supported Trisamine) at 2 mL/min for 1.5 hour. Compartment 4 is further rinsed with CH_2Cl_2, which goes into the vial.

After purification, the solution in the vial contains the N-Cbz protected product.



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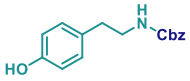
Substrate Scope

Tolerated functional groups

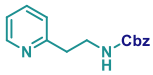
A wide range of functional groups are tolerated including alcohols and thiols, alkenes, amides, aryl halides, carbamates, esters, ethers, ketals, nitro groups, phenols, silyls and various heterocycles (imidazole, pyridine, pyrimidine, quinoline, thiophene, etc.),

Example substrate scope (from 0.8 mmol amine or amine salt)

Primary amines



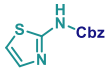
95%
in THF/H_2O (3:1)



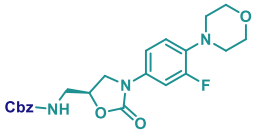
96%

90%

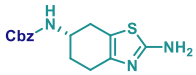
97%



22%[a]



92%



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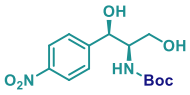

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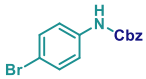
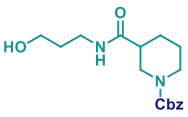


20%[a]

a) see Identified Chemistry Limitation.

Secondary amines

82%[a] (24 h)



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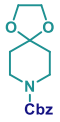

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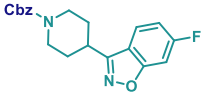

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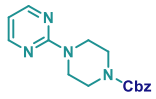
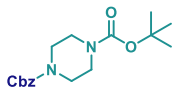
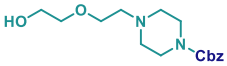


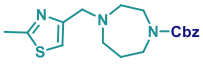
98%
99% (from 0.3 mmol)



88%



99%



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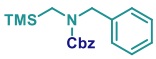

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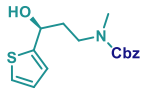

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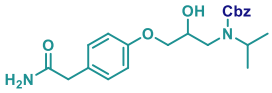

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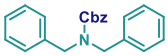

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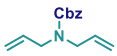
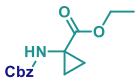


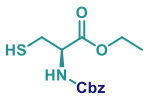
98%

a) see Identified Chemistry Limitation.

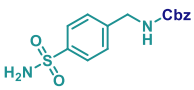
Amine salts



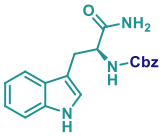
99%
(HCl salt)



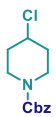
98%
(HCl salt)



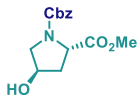
95%
(HCl salt)



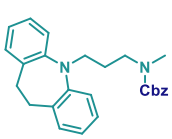
95%
(HCl salt)



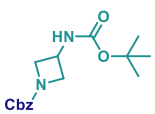
99%
(HCl salt)



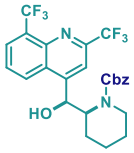
91%
(HCl salt)



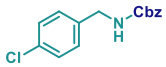
99%
(HCl salt)



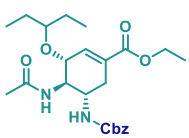
99%
(HCl salt)



92%
(HCl salt)



95%
(*p*-TsOH salt)



95%
(H_3PO_4 salt)

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Cartridge product numbers: B031, B131

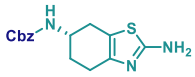
Identified Chemistry Limitation

Insoluble starting materials
Zwitterions like unprotected amino acids are poorly soluble in THF/EtOH (3:1) and THF/H_2O (3:1).

Reactivity
Sterically hindered secondary amines reacted more slowly in the Cbz protection, therefore longer reaction time is required. In these cases, unreacted amines were observed in the final solution of the crude product.

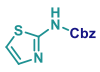
55% (3 h)
82% (24 h)

Molecules bearing two amine moieties (one alkyl amine and one aryl amine) showed full selectivity toward the alkyl amine.



99%

Additional list of identified compounds with low to no reactivity:



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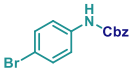

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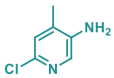

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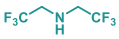

0%

Reaction Parameter Editing

Editing parameters:

Parameter 1	Reaction time of Cbz 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 THF/EtOH (3:1). If insoluble, a solution of THF/H_2O (3:1) can be use instead.
The starting amine salt shall be soluble in THF/H_2O (3:1).

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

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Cartridge product numbers: B031, B131

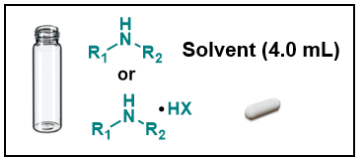
Sample Preparation

Precaution
To ensure a successful reaction in the Synple Chem synthesizer, automated CH_2Cl_2 wash shall be run before setting up a Cbz protection reaction.

Setup

Components for sample preparation:

- Vial
- Stir bar
- Alkyl amine or alkyl amine salt (up to 0.8 mmol)
- Reaction solvent (4 mL)



Guide of solvents and ratios for sample preparation

1) **Alkyl amine (0.8 mmol)**
   THF (3.0 mL, >99.9%) and EtOH (1.0 mL, >99.8%)

2) **Alkyl amine mono salt (0.8 mmol)**
   THF (3.0 mL, >99.9%) and H_2O (1.0 mL)
   N,N-Diisopropylethylamine (155 µL, 0.88 mmol, 1.1 equiv)

3) **Alkyl amine (<0.8 mmol)**
   THF (3.0 mL, >99.9%) and EtOH (1.0 mL, >99.8%)

4) **Tips for sample preparation**
   - *Sonication may help dissolving poorly soluble materials.*
   - *Pre-grinding the solid alkyl amines or alkyl amine salts may help dissolving poorly soluble materials.*
   - *For insoluble alkyl amines in THF/EtOH (3:1), THF (3.0 mL, >99.9%) and H_2O (1.0 mL) can be used as an alternative solvent system.*

Machine Solvents for the use with N-Cbz 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: –
	S3: MeOH, >99.9%
	S4: –
	S5: –

Machine Cleaning after N-Cbz Protection Reaction

1) Run automated MeOH wash after the N-Cbz Protection reaction.
2) Run automated CH_2Cl_2 wash before starting a new N-Cbz Protection reaction.

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Cartridge product numbers: B031, B131

Solvent Consumption and Run Time

SEQUENCE RUNTIME	
Reaction Sequence	**Time**
Nosyl protection	4 h 45 min

SOLVENT COMSUMPTION FOR BOC DEPROTECTION	
For Reaction Setup	**Amount**
Tetrahydrofuran (THF)	2 mL-
Ethanol (EtOH)	2 mL
Machine Solvents	
Dichloromethane (CH_2Cl_2)	31 mL

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