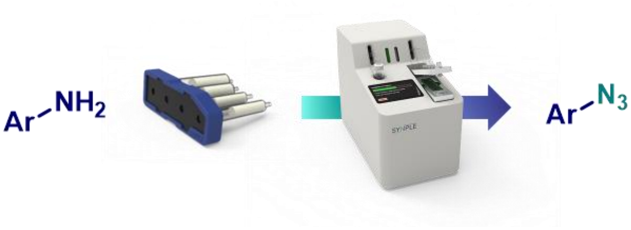
Application Note – Azide Formation (aryl amines)

Introduction

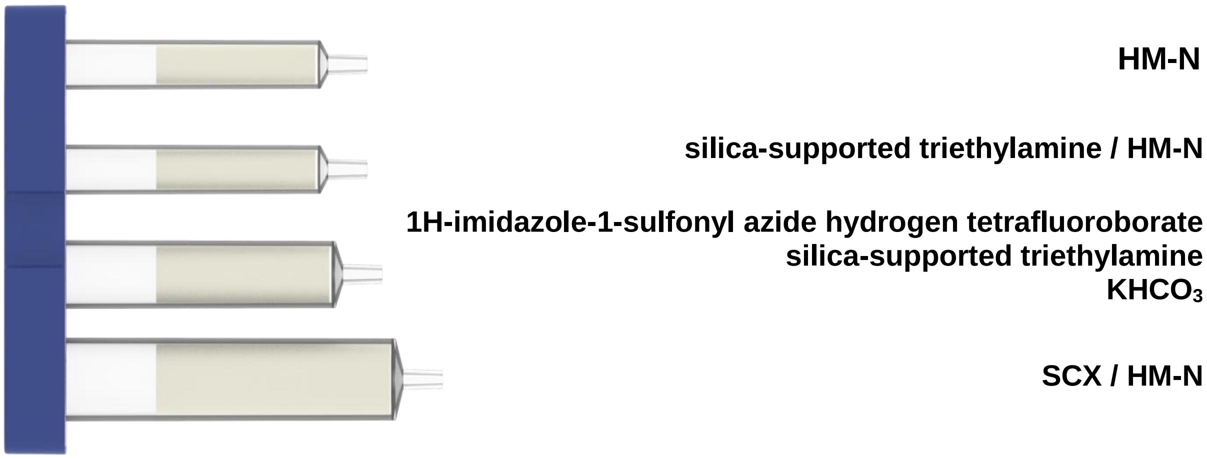
Azide formation by diazo transfer reaction of primary amines is a highly important reaction in organic chemistry and chemical biology. Azides have been widely utilized as the key reacting partner in Cu-catalyzed azide-alkyne cycloaddition (CuAAC) reaction for bioorthogonal click chemistry, peptide/protein/antibody conjugation and polymerization processes. More than a valuable functional group for derivatization, azides also show good chemical stability toward many acids and bases, so that they can serve as an ideal protecting group for primary amines, particularly in the field of carbohydrate chemistry. Commonly used diazo transfer agents include triflyl azide, fluorosulfuryl azide and 1H-imidazole-1-sulfonyl azide salts. Triflyl azide and fluorosulfuryl azide require fresh preparation from highly hazardous reagents and can only be obtained as a stock solution. On the contrary, 1H-imidazole-1-sulfonyl azide can be prepared and stored safe as its H_2SO_4 or HBF_4 salt, which enables easier handling and better control of stoichiometry against reactants. So far, these reagents are in short of commercial availability, which has largely limited the broad adoption of this azide formation chemistry.



Using the approach described in this application note, the Synple Chem synthesizer offers an easy and fast automated method for the conversion of primary alkyl amines or amine salts into the corresponding azides via a diazo transfer process.

Cartridge Contents

The cartridge contains a set of reagents to carry out the azide formation on a scale of up to 0.5 mmol.

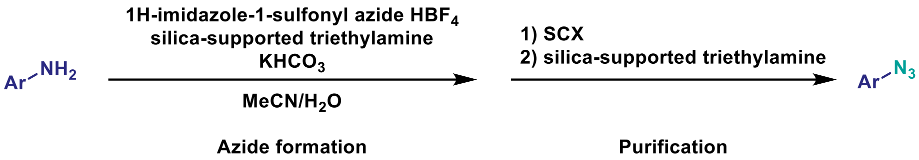


The method can be used for the following transformations:

- Azide formation from primary aryl amines.

Reaction Scheme

This section describes the general course of azide formation:



References and Publications

(1) Titz, A.; Radic, Z.; Schwardt, O.; Ernst, B. A Safe and Convenient Method for the Preparation of Triflyl Azide, and Its Use in Diazo Transfer Reactions to Primary Amines. *Tetrahedron Lett.* **2006**, *47* (14), 2383–2385. Link.

(2) Goddard-Borger, E. D.; Stick, R. V. An Efficient, Inexpensive, and Shelf-Stable Diazotransfer Reagent: Imidazole-1-Sulfonyl Azide Hydrochloride. *Org. Lett.* **2007**, *9* (19), 3797–3800. Link.

(3) Fischer, N.; Goddard-Borger, E. D.; Greiner, R.; Klapötke, T. M.; Skelton, B. W.; Stierstorfer, J. Sensitivities of Some Imidazole-1-Sulfonyl Azide Salts. *J. Org. Chem.* **2012**, *77* (4), 1760–1764. Link.

(4) Meng, G.; Guo, T.; Ma, T.; Zhang, J.; Shen, Y.; Sharpless, K. B.; Dong, J. Modular Click Chemistry Libraries for Functional Screens Using a Diazotizing Reagent. *Nature* **2019**, *574* (7776), 86–89. Link.

Reaction Procedure

1) Azide formation

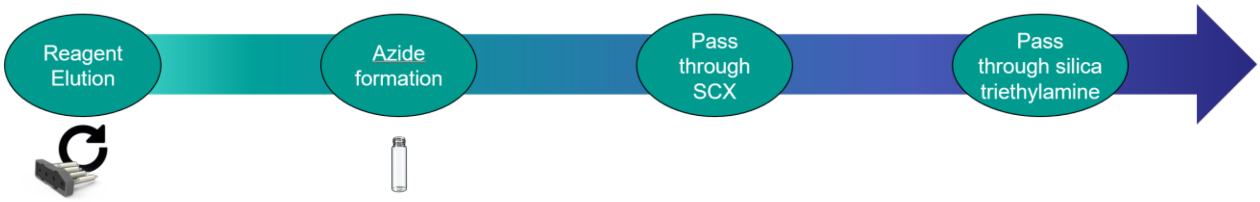
In the first step, a solution of MeCN and H_2O is added to compartment 3 at 1 mL/min. 1H-imidazole-1-sulfonyl azide hydrogen tetrafluoroborate is dissolved, neutralized over silica-supported triethylamine, and eluted together with $KHCO_3$ into the vial, which contains a solution of an aryl amine in MeCN and H_2O. The mixture is further stirred for 18 hours at 50 °C.

2) Purification

The reaction mixture is passed through compartment 4 (SCX) at 2 mL/min. Imidazole-containing byproduct is scavenged in this step. Compartment 4 is further rinsed with a solution of MeCN and H_2O, which goes into the vial.

The solution in the vial is further loaded into compartment 2 (silica-supported triethylamine) at 1 mL/min. Any possible proton leaching from SCX is scavenged in this step. Compartment 2 is further rinsed with a solution of MeCN and H_2O.

After purification, the solution in the vial contains the azide product.

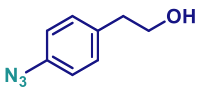


Substrate Scope

Tolerated functional groups

A wide range of functional groups are tolerated such as aliphatic alcohols, phenols, Boc-protected amines, tertiary amines, N-unprotected indoles, esters, amides, carbamates, acetals and heterocycles (imidazole, pyridine, etc.).

Example substrate scope (from 0.5 mmol aryl amine)



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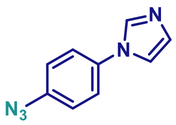

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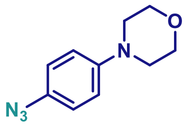

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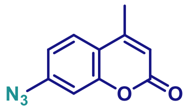

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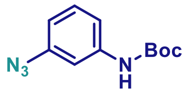

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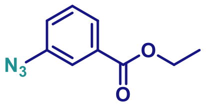

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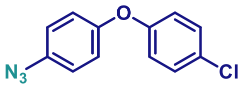

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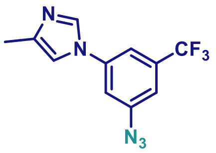

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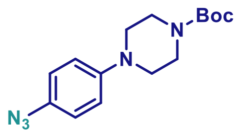

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Identified Chemistry Limitation

Stoichiometry

At present, the cartridge is designed to convert substrates containing *mono* primary amino group into the corresponding azides. When using starting materials containing two possible reaction sites, a mixture of products can be observed (mono- and diazidated products).

Basic functional groups

When amines or amine salts contain a basic moiety, such as trialkylamine, imidazole, pyridine and aniline, SCX purification would trap the azide product. By disabling the SCX purification step, all formed azide product would be obtained in the solution in the vial (see Substrate Scope).

Alternatively, we suggest the following operations:

1) **without workup**
   The crude mixture is concentrated and purified by column chromatography.
2) **workup**
   The crude mixture in MeCN and H_2O is phase-separated with EtOAc (40 mL). The aqueous phase is extracted with EtOAc (20 mL). The combined organic phase is washed with H_2O (10 mL), brine (10 mL), dried over Na_2SO_4 and concentrated. The residue is purified by column chromatography.

Acid labile functional groups

If amines or amine salts contain an acid labile functional group, such as acetals, the formed azide products may decompose over SCX. By disabling the SCX purification step, all formed azide product would be obtained in the solution in the vial (see Substrate Scope).

Carboxylic acids

When amines or amine salts contain a carboxylic acid group, the reaction proceeds smoothly giving azide products in a comparable yield as non-acidic substrates. Due to the high polarity of the azido carboxylic acid products and occasional formation of precipitation, it often leads to a low recovery of the product after the standard purification. By disabling the SCX purification step, all formed azido carboxylic acid product would be obtained in the solution in the vial (see Substrate Scope).

Competing reactions

A tiny amount of secondary sulfamide (R-NH-SO_2-NH_2) could be observed as the side product, which can be easily separated due to its much higher polarity comparing to the one of the azide product. If amines or amine salts contain a secondary amine, tertiary sulfamide may form during the reaction leading to an overall lower yield.

Reaction Parameter Editing

Editing parameters:

Parameter 1	Reaction time for Azide Formation (seconds)

Enabling and Disabling parts:

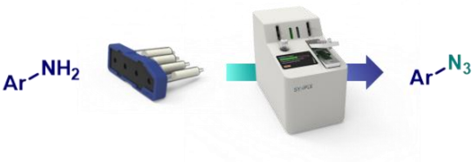
Part 1: Purification step
Purification step of the sequence can be disabled. In case of substrates bearing basic functional groups and carboxylic acids the purification might not be suitable (see Identified Chemistry Limitation). The synthesizer will then provide the crude product in solution in the vial after the azide formation step. A manual aqueous extraction can be performed to remove water in the crude mixture.

Reaction Planning

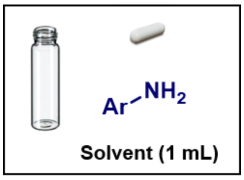
Solubility of reactants
Aryl amines shall be soluble in the reaction solvents (MeCN and H_2O).

Tolerance of air and/or moisture
Azide formation reaction using Synple Chem synthesizer is insensitive toward air or moisture.

Sample Preparation



Precaution
To ensure a successful reaction in the Synple Chem synthesizer, the last automated wash shall be done with MeOH before setting up an azide formation reaction.



Setup
Components for sample preparation:

- Vial
- Aryl amine salt (0.5 mmol)
- Stirbar
- Solvent (1.0 mL)

Guide of solvents and ratios for sample preparation:

1) **Aryl amines**
   MeCN (0.5 mL, 99.9%) and H_2O (0.5 mL, deionized)

Machine solvents for the use with Azide Formation cartridges

The following solvents are connected to the color-coded lines.

🟩	S1: –
🟦	S2: EtOAc, HPLC grade
🟥	S3: MeOH, >99.8%
🟨	S4: –
🟧	S5: MeCN/H_2O (v/v = 1/1)

Machine Cleaning after Azide Formation Reaction

1) Run automated MeOH wash after the azide formation reaction.

Miscellaneous

Tips for reaction monitoring
Azides can give different performance in mass spectroscopy. When the reaction is monitored by LC-MS, it is possible to see the molecular mass of the azide product, while sometimes *only* the fragmented mass $[M-N_2+H]^+$ is observed. Mostly azide products have much lower polarity so that they are easily detectable by TLC.

Solvent Consumption and Run Time

SEQUENCE RUNTIME	
Reaction Sequence	**Time**
Azide formation (aryl amines)	19 h 43 min

SOLVENT COMSUMPTION FOR BOC DEPROTECTION	
For Reaction Setup	**Amount**
Acetonitrile (ACN)	0.5 mL
Water (H_2O)	0.5 mL
Machine Solvents	
Acetonitrile (ACN) – water (H_2O) mixture (1:1)	27 mL
EtOAc	59 mL