Application Note – N-Heterocycle Formation

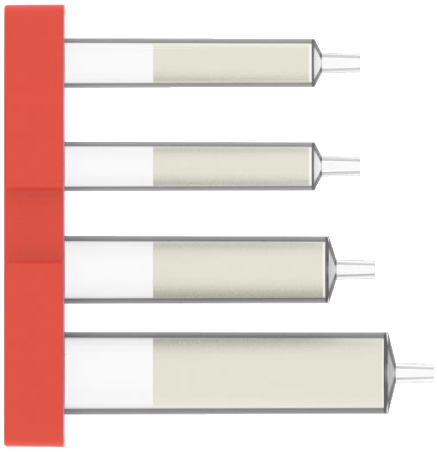
Introduction

Saturated N-heterocycles are a class of highly interesting and desirable structural moieties in the research & development of novel pharmaceuticals since their three-dimensional topology can significantly broaden the existing chemical space, providing additional opportunities to tune both physical and chemical properties of the target molecules. However, the preparation of these saturated N-heterocycles is less straightforward. One novel synthetic protocol based on the usage of SnAP (stannyl amine protocol) reagents has recently been developed in Professor Jeffrey Bode's laboratory, allowing rapid construction of substituted morpholines, piperazines, oxazepanes, diazepanes, spirocyclic and many other attractive saturated scaffolds. As one of the most versatile approaches to date, SnAP chemistry has been well recognized and widely used by drug discovery groups in pharmaceutical and biotech industries. SnAP chemistry enjoys many advantages including the structural diversity of SnAP reagents, an exceptionally broad substrate scope, and the fact that a standard set of reaction conditions is used regardless of the substrate. Therefore, SnAP chemistry shows good potential in forming a large number of different "flavors" of saturated N-heterocyclic products, which is particularly ideal for building compound libraries. Additionally, the unprotected nitrogen in the products can be diversified directly granting further expansion of the structural complexity. Admittedly, SnAP chemistry, like most organic reactions, requires several labor intensive steps, including weighing of the reagents, prior complexation of copper catalyst and ligand, preparation of an imine intermediate, a multi-step aqueous workup, and final purification by column chromatography. The usage of toxic tin reagents also makes the reaction handling difficult and undesirable.

Using the approach described in this application note, the Synple Chem synthesizer offers an easy and fast automated method for the synthesis of substituted, saturated N-heterocycles from aldehydes based on SnAP chemistry.

Cartridge Contents

The cartridge contains a set of reagents to carry out the N-hetereocycle formation on a scale up to 0.5 mmol. For all available N-heterocycle formation cartridges the contents are identical, with the exception of the SnAP reagent, which varies depending on the type of N-heterocycle to be formed.



immobilized SnAP Reagent
4 Å molecular sieves

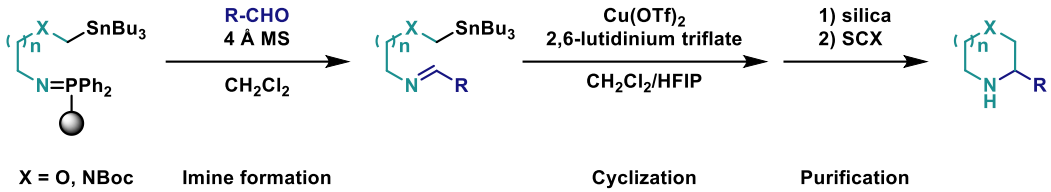
2,6-lutidinium triflate
$Cu(OTf)_2$

SCX

silica

Reaction Scheme

This section describes the general course of the N-heterocycle formation (SnAP):



The SnAP chemistry for the synthesis of saturated N-heterocycles consists of four steps:

1) **Imine formation**
The starting aldehyde reacts with the SnAP reagent (immobilized on a triphenylphosphine resin) in anhydrous CH_2Cl_2. The triphenylphosphine oxide byproduct remains on the resin.

2) **Cyclization**
Cu-mediated cyclization utilizes a preformed $Cu(OTf)_2$/2,6-lutidine complex in anhydrous HFIP (1,1,1,3,3,3-hexafluoroisopropanol).

3) **Work up**
The standard basic aqueous workup to remove copper salts is replaced in the automated sequence by filtering the reaction solution through a plug of silica.

4) **Purification:** In the automated sequence the filtrate is subjected to a catch and release purification process using SCX.

References and Publications:

(1) Vo, C. V. T.; Mikutis, G.; Bode, J. W. SnAP Reagents for the Transformation of Aldehydes into Substituted Thiomorpholines - An Alternative to Cross-Coupling with Saturated Heterocycles. *Angew. Chem. Int. Ed.* **2013**, *52* (6), 1705–1708. Link.

(2) Vo, C.-V. T.; Luescher, M. U.; Bode, J. W. SnAP Reagents for the One-Step Synthesis of Medium-Ring Saturated N-Heterocycles from Aldehydes. *Nat. Chem.* **2014**, *6* (4), 310–314. Link.

(3) Luescher, M. U.; Geoghegan, K.; Nichols, P. L.; Bode, J. W. SnAP Reagents for a Cross-Coupling Approach to the One-Step Synthesis of Saturated N-Heterocycles. *Aldrichim. Acta* **2015**, *48* (2), 43–48. Link.

(4) Lovering, F.; Bikker, J.; Humblet, C. Escape from Flatland: Increasing Saturation as an Approach to Improving Clinical Success. *J. Med. Chem.* **2009**, *52* (21), 6752–6756. Link.

Reaction Procedure

1) Imine formation

In the first step, anhydrous CH_2Cl_2 (4.5 mL) is loaded by the synthesizer and dosed through compartment 1 (immobilized SnAP reagent and 4 Å molecular sieves) at 1 mL/min into the vial to dissolve the neat aldehyde. The solution is then circulated through compartment 1 at 1 mL/min for the designated time (see table below), while compartment 1 is heated at 50 °C and the vial is heated at 35 °C. After the imine formation is complete, compartment 1 is rinsed with anhydrous CH_2Cl_2 (3.5 mL), which goes into the vial.

N-Heterocycle cartridge	Time for imine formation (full sequence)
Morpholine	6 h
Oxazepane	6 h
Piperazine	6 h
Diazepane	6 h
2-Methylmorpholine	6 h

N-Heterocycle cartridge	Time for imine formation (full sequence)
3-Methylmorpholine	16 h
Morpholine-2-spiro-(3-Pyr)	9 h
Morpholine-2-spiro-(4-Pip)	9 h
9-OMe-Benzoxazepane	9 h
7-Br-9-OMe-Benzoxazepane	9 h

2) Cyclization

Anhydrous HFIP (2.0 mL) is loaded by the synthesizer and dosed through compartment 2 [$Cu(OTf)_2$ and 2,6-lutidinium triflate] into the vial. The solution of imine in anhydrous CH_2Cl_2 and HFIP is circulated through compartment 2 at 2 mL/min for the designated time (see table below), while compartment 2 is heated at 40 °C and the reaction vial is heated at 37 °C. After cyclization, compartment 2 is rinsed with anhydrous CH_2Cl_2 (2.0 mL), which goes into the vial.

N-Heterocycle cartridge	Time for cyclization (full sequence)
Morpholine	3 h
Oxazepane	3 h
Piperazine	3 h
Diazepane	3 h
2-Methylmorpholine	3 h

N-Heterocycle cartridge	Time for cyclization (full sequence)
3-Methylmorpholine	3 h
Morpholine-2-spiro-(3-Pyr)	4 h
Morpholine-2-spiro-(4-Pip)	4 h
9-OMe-Benzoxazepane	3 h
7-Br-9-OMe-Benzoxazepane	3 h

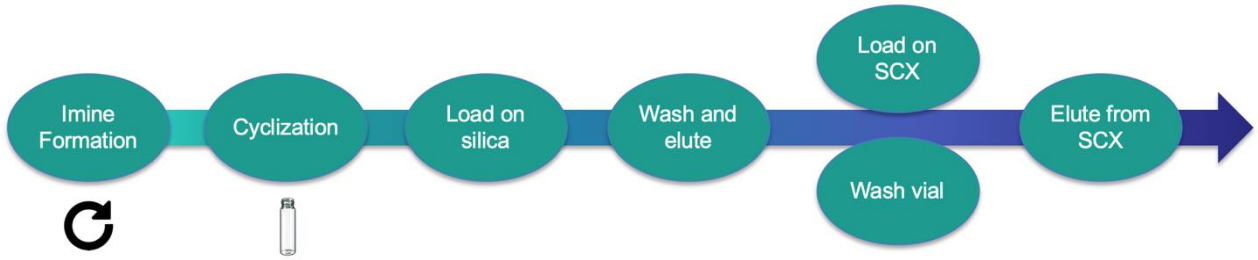
3) Purification

The reaction mixture is loaded into compartment 4 (silica) at 2 mL/min. Most of the Cu salt is removed in this step. Compartment 4 is further rinsed with MeOH and the filtrate is loaded into compartment 3 (SCX) at 2 mL/min. Compartment 3 is further rinsed with MeOH and the filtrate, which contains all the non-basic substances (formed N-heterocycles are basic and therefore trapped by SCX), is discarded to waste.

4) Product release

Compartment 3 (SCX) is rinsed with 2.5 M *N*,*N*-diisopropylamine in THF, which goes into the vial.

After product release, the solution in the vial contains the N-heterocycle product.



Substrate Scope

Tolerated functional groups

A wide range of functional groups are tolerated such as halides, esters, nitriles, boronic esters, carbamates, protected amines and various heterocycles (pyridine, quinoline, pyrimidine, triazole, thiophene, piperidine, etc.). In general, functional groups that are compatible with conditions of radical chemistry are tolerated.

Trends for aldehyde substrates

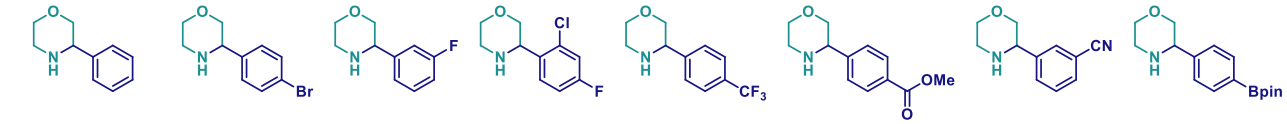
Generally, higher yields are obtained for reactions with aromatic, electron-deficient aldehydes. Yields from electron-rich aldehydes tend to be decline due to the lower yield of the imine formation step.

Reactions with aliphatic aldehydes give lower yields as the cyclization is less efficient hampered by the potential enamine tautomerization.

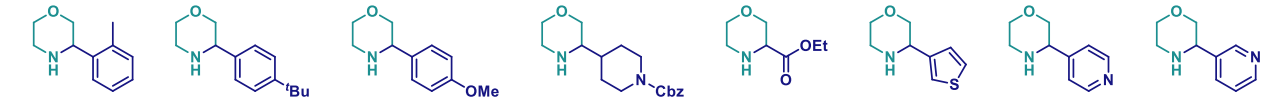
Combinations of aldehydes and sterically hindered SnAP reagents, such as spirocyclic morpholines, 2-methyl and 3-methylmorpholines, can also result in lower yields.

Example substrate scope (from 0.5 mmol aldehyde)

Morpholines

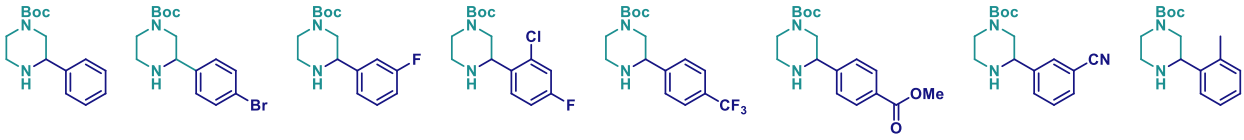


60%
45% (0.2 mmol CHO)
64%
53%
45%
46%
50%
62%
27%

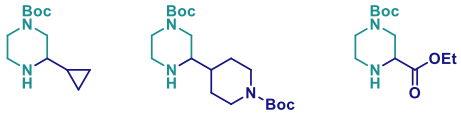


42%
33%
15%
36% (0.4 mmol CHO)
38%
45%
44%
33%

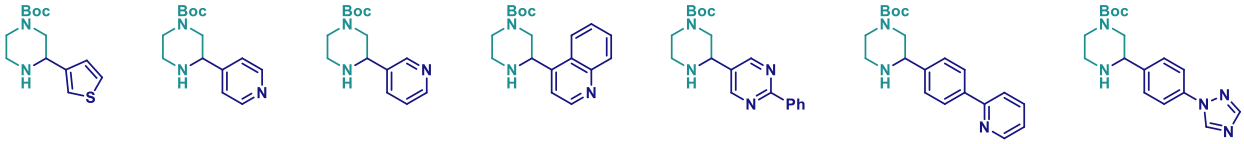
Piperazines



50%
70%
64%
64%
54%
57%
59%
42%

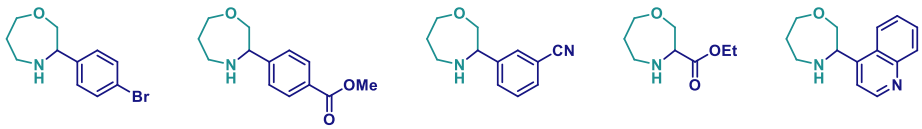


44%
33%
56%



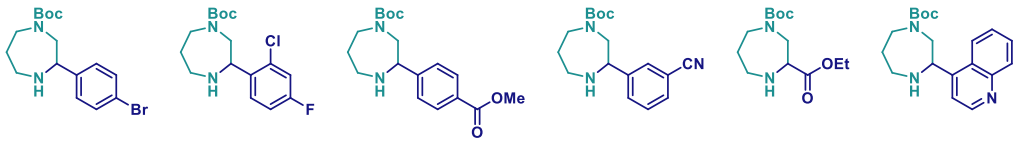
62%
57%
61%
72%
67%
68%
75%

Oxazepanes



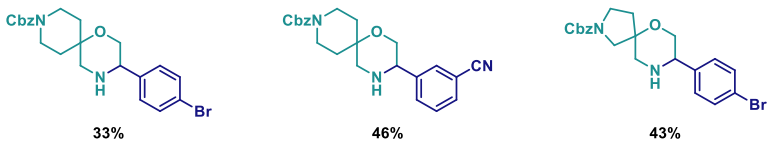
69%
69%
70%
23%
67%

Diazepanes



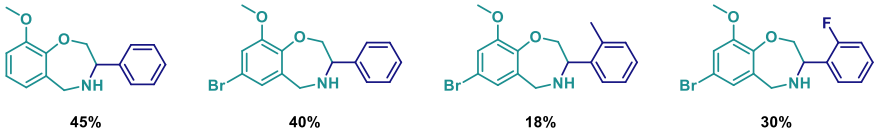
56%
61%
51%
72%
37%
60%

2-Spirocyclic morpholines

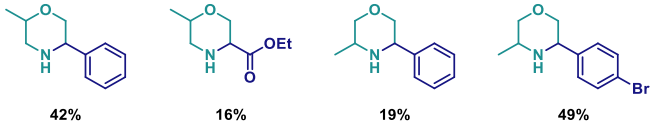


33%
46%
43%

Substituted benzoxazepanes



Methylmorpholines



Identified Chemistry Limitation

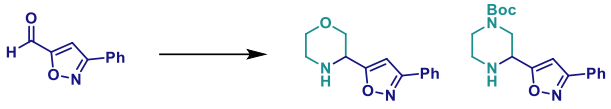
Reactants

At present, the reaction has not been optimized for ketones, from which the ketimine formation is very slow with the current setup. However, it is possible to pre-form the ketimine in batch, then place it into the synthesizer to run the rest of the reaction (cyclization and purification) automatically. For this alternative cartridge, please contact us at info@synplechem.com.

Aldehydes containing *β*-heteroatoms

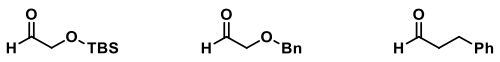
Aldehydes containing a coordinating heteroatom at the *β*-position of the formyl group do not lead to the formation of N-heterocyclic products. The generated imine acts as a bidentate ligand that complexes the Cu-catalyst, which consequently stops the cyclization completely and/or leads to fast decomposition of the imine intermediate.

When oxygen is located at the *β*-position of the formyl group in the aldehyde, the N-heterocyclic product is formed smoothly (see Substrate Scope, ethyl glyoxylate). Additional list of compatible O-containing aldehydes:



Aliphatic aldehydes

Acyclic aliphatic aldehydes are not tolerated in the reaction, most likely due to facile enamine tautomerization leading to rapid decomposition of the imine intermediate.

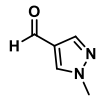


However, *α*,*α*-disubstituted (cyclic) aliphatic aldehydes could lead to formation of the N-heterocyclic products, albeit in lower yields (see Substrate Scope, 1-Boc/Cbz-piperidine-4-carboxaldehyde and cyclopropyl-aldehyde).

Electron-rich aldehydes

In general, highly electron-rich aldehydes are not suitable starting materials for N-heterocycle formation (based on SnAP chemistry) for the following reasons:

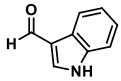
1) the imine formation is usually in poor yields with the current setup on the synthesizer.



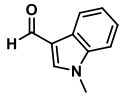
<=20%
(piperazine)

2) the aldehyde and/or the imine may be readily oxidized by Cu(II) leading to uncontrollable side reactions and/or decomposition.

no product



no product

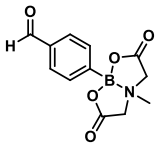


no product

Non-tolerated functional groups
Aldehydes containing carboxylic acid groups are not tolerated because of their interference with both imine formation and cyclization steps.

Solubility
Aldehydes shall be soluble in CH_2Cl_2 in order to be circulated through the immobilized SnAP reagent for the imine formation. CH_3CN may be used as co-solvent (up to 1.0 mL), but slightly lower yields would be obtained. Insoluble aldehydes may cause damage to the synthesizer.

List of insoluble aldehydes in CH_2Cl_2



(4-boryl-MIDA)-benzaldehyde

See section “Reaction Parameter Editing” to disable the automatic solvation of the substrate with CH_2Cl_2.

Acetal formation
For a few cases of using N-containing aldehydes, such as pyridyl and quinonyl aldehydes, methyl acetal formation of the residual aldehyde can be observed during the SCX purification, which would present in the crude product. This requires further purification, but the yield of the desired products remains unchanged.

Boc deprotection
In rare cases, up to 20% Boc deprotection can be observed. This can be avoided by disabling the SCX purification step (see Reaction Parameter Editing).

Reaction Parameter Editing

Editing parameters

Parameter 1	Imine formation temperature of cartridge (°C)
Parameter 2	Imine formation temperature of reaction vial (°C)
Parameter 3	Imine formation reaction time (seconds)
Parameter 4	Cyclization temperature of cartridge (°C)
Parameter 5	Cyclization temperature of vial (°C)
Parameter 6	Cyclization reaction time (seconds)

Enabling and Disabling parts

Part 1
Substrate solvation step

This can be disabled if the user prefers to dissolve the aldehyde substrate him-/herself instead of loading it crude into vial. For dissolving use 4.5 mL of dry CH_2Cl_2. Other amounts or type of solvents can lead to lower conversion. It is possible to use Acetonitrile instead as solvent but slightly lower yields will be obtained.

Part 2:
Purification step

The purification step of the reaction sequence can be disabled. In case of substrates containing highly acid sensitive functional groups, the purification might not be suitable. The synthesizer will then provide the N-heterocyclic product in the solution in the vial after the cyclization step.

For a manual workup before column purification, the following steps are recommended.

1) dilute the reaction mixture with CH_2Cl_2 (10 mL).
2) wash the mixture with commercial 25% aqueous ammonia (3 mL × 2) to remove the copper.
3) wash the combined organic phase with deionized H_2O (3 mL × 2).
4) dry over Na_2SO_4, filter off and concentrate to dryness.

Reaction Planning

Solubility of reactants
Aldehydes shall be soluble in the reaction solvent (CH_2Cl_2). CH_3CN may be used as co-solvent (up to 1.0 mL), but slightly lower yields would be obtained.

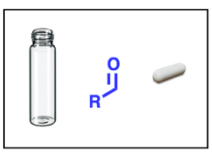
Tolerance of air and/or moisture
N-heterocycle formation using Synple Chem synthesizer is sensitive toward air and moisture. Therefore, CH_2Cl_2 and HFIP shall be properly treated before using (see Sample Preparation for solvent specification).

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 N-heterocycle formation reaction.

Setup
Components for sample preparation:

- Vial
- Aldehyde (0.5 mmol)
- Stirbar
- No solvent



Machine Solvents for the use with SnAP cartridges

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

	S1: CH_2Cl_2, 99.8%, anhydrous, 50 ppm amylene stabilized
	S2: 1,1,1,3,3,3-hexafluoroisopropanol, 99.9%, anhydrous and distilled Available from Synple Chem
	S3: MeOH, >99.9%
	S4: *N*,*N*-diisopropylamine (175 mL, >=99.5%) in THF (325 mL, >99.5%, BHT stabilized)
	S5: –

Machine Cleaning after N-Heterocycle Formation Reaction

1) Run automated CH_2Cl_2 wash after the N-heterocycle formation reaction.

Solvent Consumption and Run Time

SEQUENCE RUNTIME	
Reaction Sequence	**Time**
Full Heterocycle sequence (*available for Morpholine, Oxazepane, Piperazine, Diazepane*)	11 h 50 min
Short Heterocycle sequence (*available for Morpholine, Oxazepane, Piperazine, Diazepane*)	5 h 36 min
3-Methylmorpholine	21 h 50 min
2-Methylmorpholine	14 h 50 min
Morpholine-2-spiro-(3-Pyr)	15 h 52 min
Morpholine-2-spiro-(4-Pip)	15 h 52 min
Benzoxazepane	15 h 52 min
9-OMe-Benzoxazepane	14 h 50 min
7-Br-9-OMe-Benzoxazepane	14 h 50 min

SOLVENT COMSUMPTION FOR ALL HETEROCYCLE REACTIONS	
For Reaction Setup	**Amount**
no solvent	
Machine Solvents	
Dichloromethane (CH_2Cl_2)	102 mL
Hexafluoroisopropanol (HFIP)	4 mL
Methanol (MeOH)	87 mL
Diisopropylamine (DIPA) – Tetrahydrofuran (THF) mixture (13:7)	26 mL