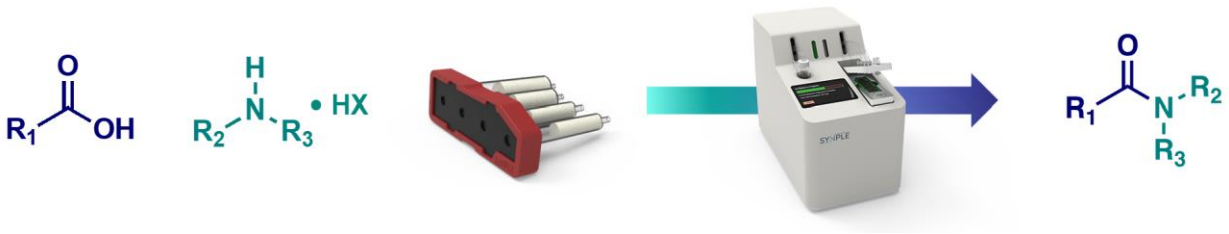
Application Note – Amide Formation (Amine Salts)

Introduction

Amide (carboxamide) group is one of the most important functionalities broadly occurring in small molecules, peptides, proteins and various natural/synthetic polymers. In pharmaceutical industry, amide formation covers nearly 25% of the patent reactions, thus presents as the most widely used synthetic method in all phases of drug discovery. Amide formation between a carboxylic acid and an amine initiated with the pre-activation of the carboxylic acid with a coupling reagent to form an active intermediate, such as acyl halides, acid anhydrides and active esters, which undergoes a nucleophilic replacement of the amine to form the amide product. Numerous coupling reagents, including carbodiimides, aminium/uronium salts, phosphonium salts, organophoporous reagents, acylazoles and other heterocycle-incorporated reagents, have been developed and become readily available from commercial vendors. Amide formation reactions are mostly run in a polar aprotic solvent, such as dichloromethane, acetonitrile and *N*,*N*-dimethylformamide. Before purification, a manual workup is usually required to remove the water-soluble byproduct of the coupling reagents, which can be lengthy and tedious. More recently, increased concerns over the coupling reagents as immune sensitizers have arisen, which prompted research in finding safer alternatives and/or improving existing protocols to meet the elevating safety standards in laboratories.

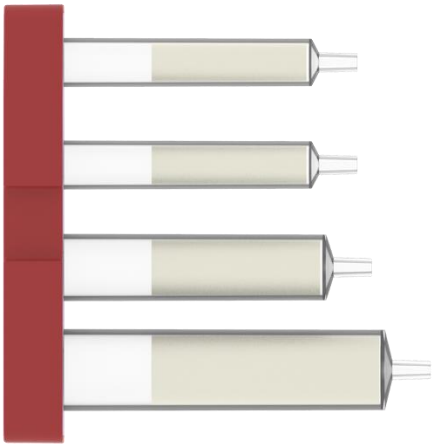
Using a solid-supported coupling reagent for amide formation has emerged as a suitable and welcoming solution in both batch and flow setup for minimizing the exposure to sensitizing chemicals, while reaction workup and purification of the amide product are also largely simplified, as the byproduct of the coupling reagent remains covalently bound to the solid support.



Using the approach described in this application note, the Synple Chem synthesizer offers an easy and fast automated method for the amide formation between a carboxylic acid and an amine salt.

Cartridge Contents

The cartridge contains a set of reagents to carry out the amide formation (salt) on a scale up to 0.5 mmol.



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SCX

Oxyma Pure
solid-supported carbodiimide

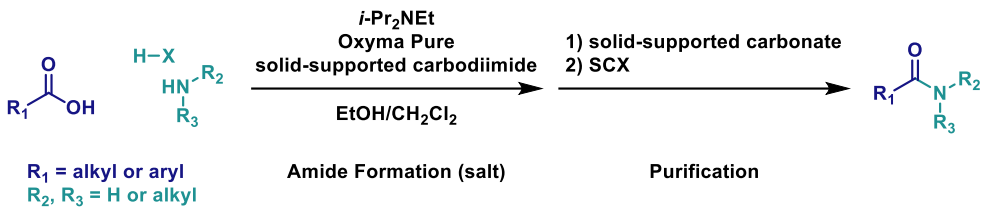
solid-supported carbonate

The method can be used for the following transformation:

- Amide formation between a carboxylic acid and an alkyl amine salt.
  (For amide formation between a carboxylic acid and a free alkyl amine, see Amide Formation)

Reaction Scheme

This section describes the general course of amide formation (salt):



References and Publications:

(1) Montalbetti, C. A. G. N.; Falque, V. Amide Bond Formation and Peptide Coupling. *Tetrahedron* **2005**, *61* (46), 10827–10852. Link.

(2) Valeur, E.; Bradley, M. Amide Bond Formation: Beyond the Myth of Coupling Reagents. *Chem. Soc. Rev.* **2009**, *38* (2), 606–631. Link.

(3) Joullié, M. M.; Lassen, K. M. Evolution of Amide Bond Formation. *Arkivoc* **2010**, 2010 (viii), 189–250. Link.

(4) McKnelly, K. J.; Sokol, W.; Nowick, J. S. Anaphylaxis Induced by Peptide Coupling Agents: Lessons Learned from Repeated Exposure to HATU, HBTU, and HCTU. *J. Org. Chem.* **2020**, *85* (3), 1764–1768. Link.

Reaction Procedure

1) Amide formation

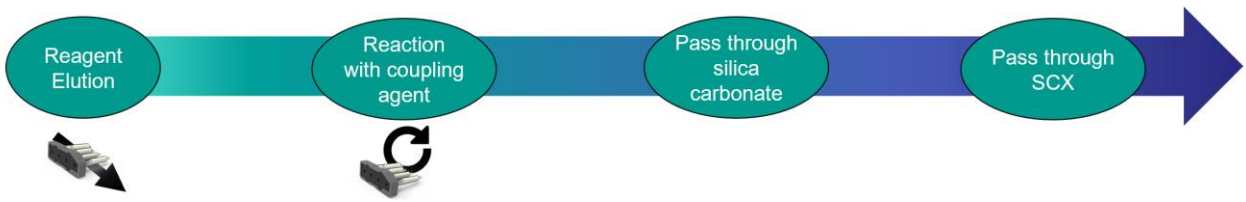
In the first step, a pre-mixed solution of an carboxylic acid, an alkyl amine salt and *N*,*N*-diisopropylethylamine in anhydrous EtOH and CH_2Cl_2 is loaded into compartment 3 to dissolve Oxyma Pure and further passed through solid-supported carbodiimide at 1 mL/min. Continuous circulation through solid-supported carbodiimide lasts for 6 hours at room temperature. When the reaction is complete, compartment 3 is rinsed with anhydrous CH_2Cl_2, which goes into the vial.

2) Purification

The reaction mixture is loaded into compartment 4 (solid-supported carbonate) at 1 mL/min. Oxyma Pure and excess amount of carboxylic acid are scavenged in this step. Compartment 4 is further rinsed with MeOH, which goes into the vial.

The solution in the vial is further loaded into compartment 2 (SCX) at 1 mL/min. Unreacted amine and *N*,*N*-diisopropylethylamine is scavenged in this step. Compartment 2 is further rinsed with MeOH, which goes into the vial.

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



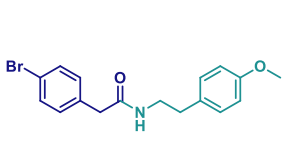
Substrate Scope

Tolerated functional groups

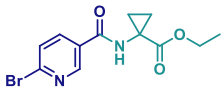
A wide range of functional groups are tolerated, such as unprotected alcohols, ketones, esters, carbamates, protected amines, nitriles, boronic esters, alkyl and aryl halides, and various heterocycles (pyridine, indole, imidazole, isoxazole, thiazole, etc.).

Example substrate scope (from 0.5 mmol alkyl amine HCl salt, unless otherwise specified)

Primary amine salt (reaction time is 3 hours):



75%
80% (from *p*-TsOH salt)



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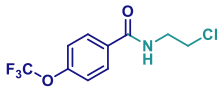
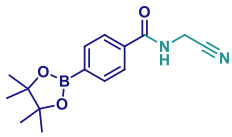


78%
75% (from 0.15 mmol)



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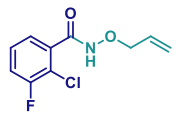

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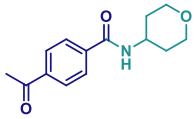

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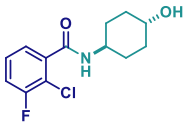

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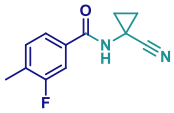
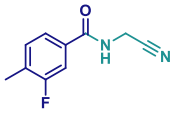


24%[a]



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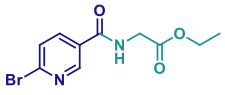

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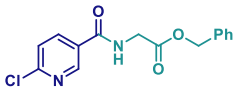

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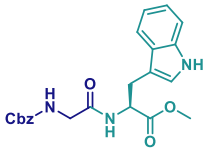

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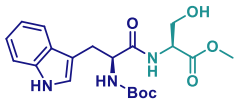

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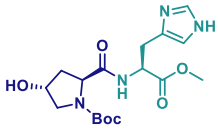
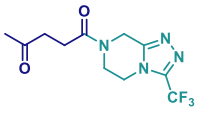


55%[a] (from 2HCl salt)

a) see Identified Chemistry Limitation.

Secondary amine salt (reaction time is 6 hours):



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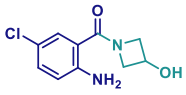

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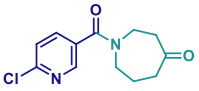

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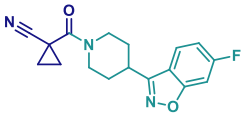
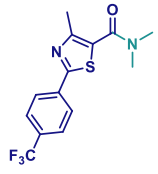


60%
64% (from *p*-TsOH salt)



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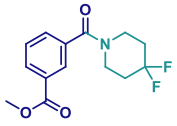

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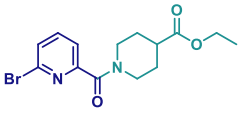

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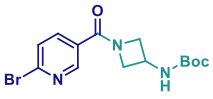

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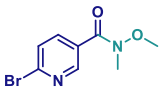
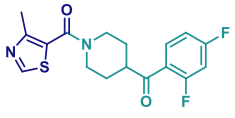


70%
(Weinreb amide)



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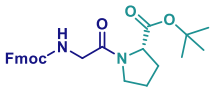


86%

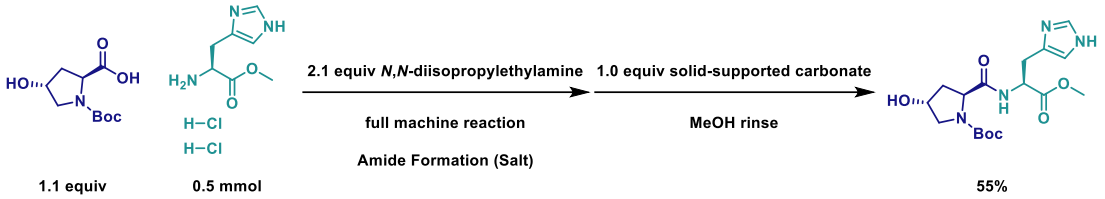
Identified Chemistry Limitation

Reactants

At present, the reaction has not been optimized for weakly nucleophilic amine salts, e.g. aryl amine salts.

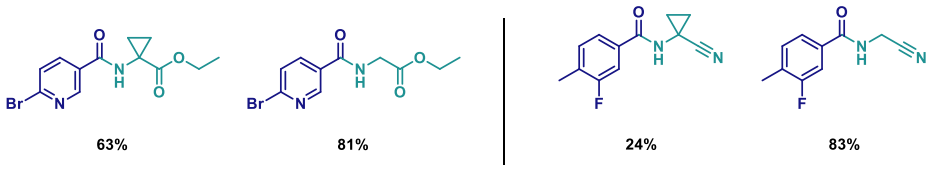
Stoichiometry

The purification of Amide Formation (Salt) reaction is optimized for alkyl amine mono salts, such as HCl and *p*-TsOH salt. When multiple salt is used, more *N*,*N*-diisopropylethylamine is needed, as shown in the following example. After the full machine reaction, the crude mixture is manually passed through additional amount of Si-CO_3 (1.0 equiv) to remove *N*,*N*-diisopropylethylamine hydrochloride, which co-elutes with the product otherwise during a normal phase silica-based chromatography.



Reactivity

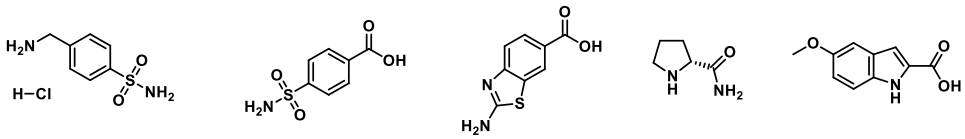
Sterically hindered amine salts react more slowly in the amide formation, therefore longer reaction time can be used to improve conversion and yield. In these cases, unreacted amines may be observed in the final solution of the crude product.



Solubility

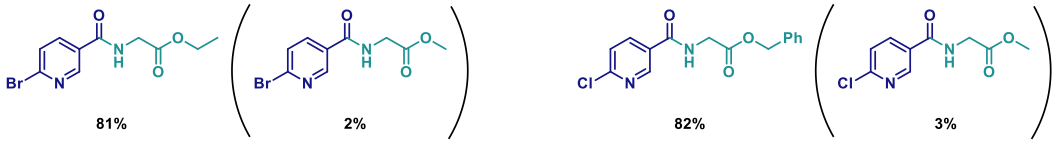
Carboxylic acid and alkyl amine salt need to be soluble in the reaction solvent of anhydrous EtOH and anhydrous CH_2Cl_2 after the addition of *N*,*N*-diisopropylethylamine. Sonication and/or pre-grinding the amine salts may help to dissolve starting materials. Addition of H_2O (up to 5.0 equiv) may be helpful as well. Insoluble materials may cause damage to the synthesizer.

List of identified compounds with poor solubility in reaction solvents:



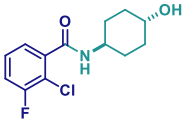
Transesterification

For amide products containing ester groups, side products via transesterification are observed occasionally, but in tiny amount.



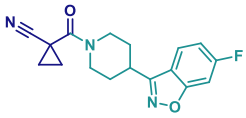
Comparison in Amide Formation using free alkyl amines and alkyl amine salts

For the same product, the reaction starting with free alkyl amines often gives high conversion and yield. It has also been observed that in alkyl amine salts, the ratio between amine and acid is sometimes inconsistent. Therefore, quantification of the salt composition by ^1H NMR may be necessary before starting an Amide Formation (Salt) reaction.



63% (from free amine)

32% (from amine HCl salt)



72% (from free amine)

61% (from amine HCl salt)
64% (from amine PTSA salt)

Miscellaneous
Other identified chemistry limitation, including reactivity and solubility, see the Application Note of Amide Formation.

Reaction Parameter Editing

Editing parameters:

Parameter 1	Reaction time for Amide Formation (seconds) e.g. 16 hours = 57600 seconds

Enabling and Disabling parts:

Part 1:
Purification step

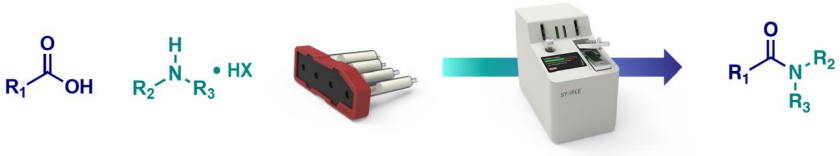
The purification step of the sequence can be disabled.

Reaction Planning

Solubility of reactants
Carboxylic acid and alkyl amine salt shall be soluble in the reaction solvents (EtOH and CH_2Cl_2) after the addition of *N*,*N*-diisopropylethylamine.

Tolerance of air and/or moisture
Amide formation (salt) reaction using Synple Chem synthesizer is insensitive toward air and moisture, although anhydrous solvents (EtOH and CH_2Cl_2) were used through the whole development. Control experiments with the addition of 2.5 mmol of H_2O (0.045 mL, 5.0 equiv based on the amine salt) gave comparable yields as the reactions without additional H_2O.

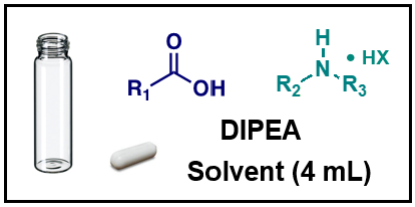
Sample Preparation



Precaution
To ensure a successful reaction in the Synple Chem synthesizer, automated CH_2Cl_2 wash should be run before setting up an Amide Formation (Salt) reaction.

Setup
Components for sample preparation:

- Vial
- Stirbar
- Alkyl amine salt and carboxylic acid



- *N*,*N*-diisopropylethylamine
- Anhydrous EtOH and anhydrous CH_2Cl_2

Guide of solvents and ratios for sample preparation

1) **Alkyl amine mono salt (0.5 mmol)**
   Carboxylic acid (0.55 mmol, 1.1 equiv)
   Anhydrous EtOH (2.0 mL, 99.5%) and anhydrous CH_2Cl_2 (2.0 mL, 99.8%, amylene stabilized)
   N,*N*-diisopropylethylamine (0.1 mL, 0.55 mmol, 1.1 equiv)

2) **Alkyl amine di salt (0.5 mmol)**
   Carboxylic acid (0.55 mmol, 1.1 equiv)
   Anhydrous EtOH (2.0 mL, 99.5%) and anhydrous CH_2Cl_2 (2.0 mL, 99.8%, amylene stabilized)
   N,*N*-diisopropylethylamine (0.18 mL, 1.05 mmol, 2.1 equiv)

3) **Alkyl amine salt (<0.5 mmol)**
   Carboxylic acid (1.1 equiv based on alkyl amine salt)
   Anhydrous EtOH (2.0 mL, 99.5%) and anhydrous CH_2Cl_2 (2.0 mL, 99.8%, amylene stabilized)
   N,*N*-diisopropylethylamine (1.1 equiv based on alkyl amine salt)

4) **Tips of sample preparation**
   - *Sonication may help dissolving poorly soluble materials.*
   - *Pre-grinding the alkyl amine salt may help dissolving poorly soluble materials.*
   - *Addition of up to 5.0 equiv H_2O may help dissolving poorly soluble materials.*

Guide of selecting sequences

1) For primary amine salts, select standard sequence (6 h). Reaction time can be shortened to 3 h (10800 s) if needed.
2) For secondary amine salts, select standard sequence (6 h).
3) For sterically hindered amine salts, reaction time can be prolonged to 16 h (57600 s, see Reaction Parameter Editing).

Machine solvents for the use with Amide Formation (Salt) cartridges
Please connect the following solvent to the color-coded solvent lines:

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

Machine Cleaning after Amide Formation (Salt) Reaction

1) Run automated MeOH wash after the Amide Formation (Salt) reaction.
2) Run automated CH_2Cl_2 wash before starting a new Amide Formation (Salt) reaction.

Solvent Consumption and Run Time

SEQUENCE RUNTIME	
Reaction Sequence	**Time**
Amide formation	3 h 50 min

SOLVENT COMSUMPTION FOR BOC DEPROTECTION	
For Reaction Setup	**Amount**
Dichloromethane (CH_2Cl_2)	2 mL
Ethanol (EtOH)	2 mL
Machine Solvents	
Dichloromethane (CH_2Cl_2)	16 mL
Methanol (MeOH)	12 mL