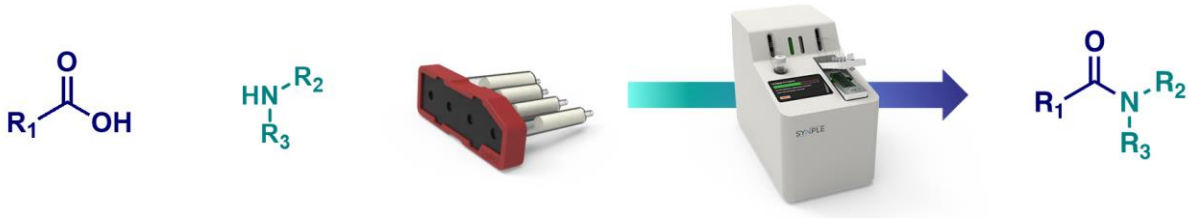
Application Note – Amide Formation (Free Amines)

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 prompt research in finding safer alternatives and/or improving existing protocols to meet the elevating safety standards in laboratories.

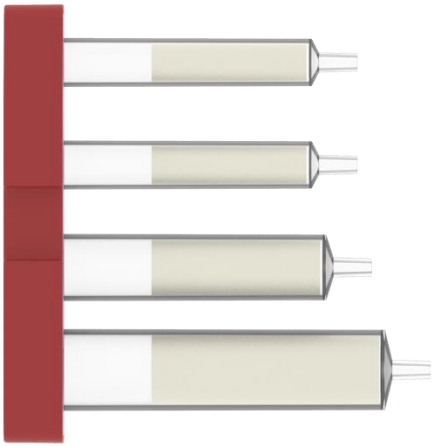
Using a solid-supported coupling reagent for amide formation has emerged as a suitable and welcoming solution in both batch and flow setup for minimizing the exposure to sensitizing chemicals, while reaction workup and purification of the amide product are also largely simplified, as the byproduct of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

Cartridge Contents

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



-

SCX (buffered)

Oxyma Pure
silica-supported carbodiimide

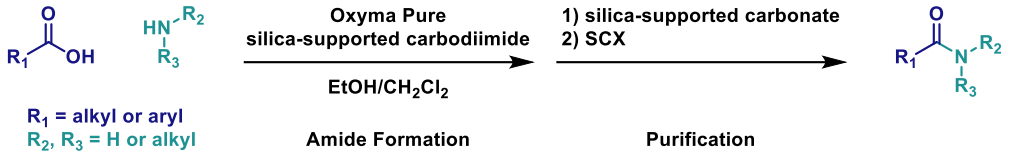
silica-supported carbonate

The method can be used for the following transformation:

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

Reaction Scheme

This section describes the general course of amide formation:



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

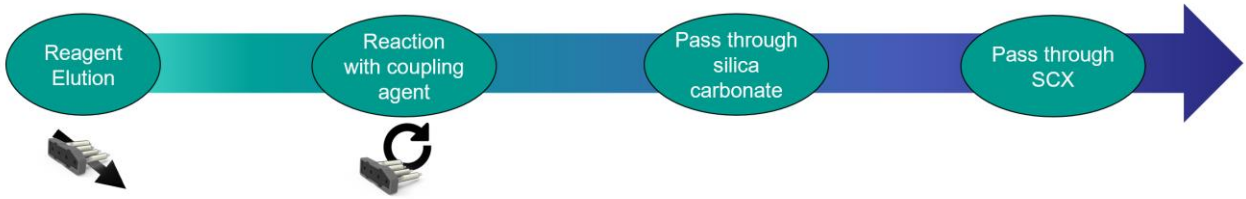
In the first step, a pre-mixed solution of carboxylic acid and free alkyl amine in anhydrous EtOH and CH_2Cl_2 is loaded into compartment 3 to dissolve Oxyma Pure and further passed through silica-supported carbodiimide at 1 mL/min. Continuous circulation of the mixture through silica-supported carbodiimide 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 (silica-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 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 amide product.



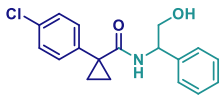
Substrate Scope

Tolerated functional groups

A wide range of functional groups are tolerated, such as unprotected alcohols, phenols, ketones, esters, carbamates, acetals, protected amines, tertiary amines, amides, azides, nitros, nitriles, hydrazides, boronic esters, alkenes, alkynes, and various heterocycles (imidazole, pyridine, thiazole, isoxazole, pyrimidine, morpholine, piperidine, piperazine, etc.).

Example substrate scope (from 0.5 mmol alkyl amine)

Primary amines without additional basic sites (reaction time is 3 hours):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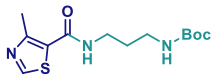
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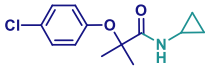
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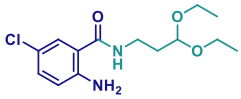
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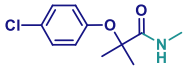
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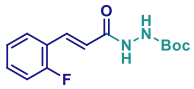
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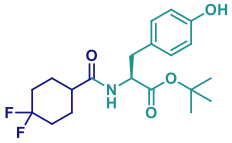
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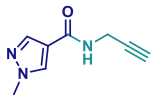
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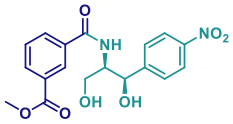
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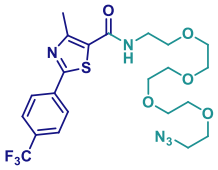
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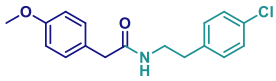
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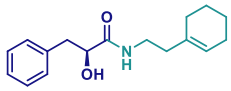
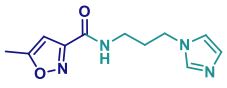

71%

a) 6 h needed (59%, 3 h).

Primary amines with additional basic sites (reaction time is 6 hours):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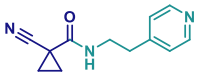
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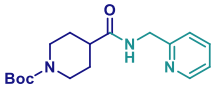
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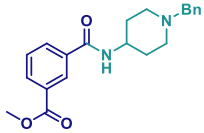
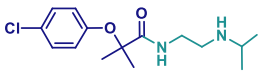

77%
91% (0.1 mmol amine)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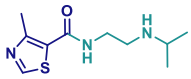
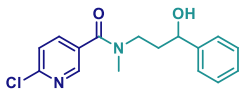

37%[a]

a) see Identified Chemistry Limitation.

Secondary amines (reaction time is 6 hours. Possible to prolong to 16 hours.):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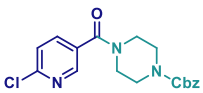
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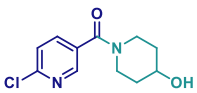
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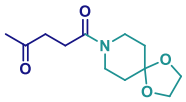
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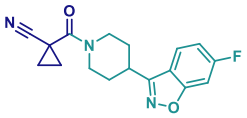
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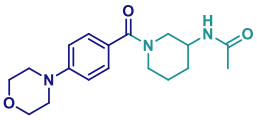
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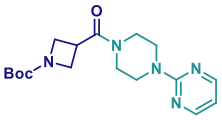
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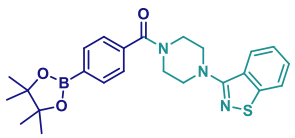
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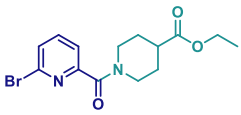
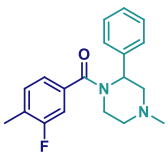
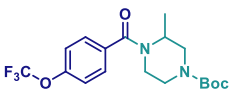
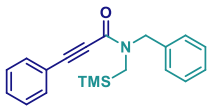

73%
91% (0.1 mmol amine)


74% (16 h)[a]


66% (16 h)[a]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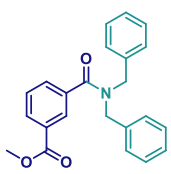
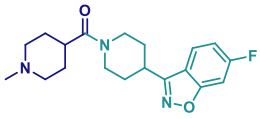

63% (16 h)[a]


36%[a]

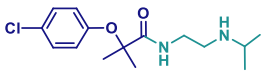
a) see Identified Chemistry Limitation.

Identified Chemistry Limitation

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

Reactivity
Sterically hindered secondary amines reacted more slowly in the amide formation, therefore longer reaction time was required (see Substrate Scope, secondary amines). In these cases, unreacted amines were observed in the final solution of the crude product.

Amines bearing two amine moieties (one unhindered and one hindered) showed much higher selectivity toward the amide formation of the unhindered amine moiety, though the yields were moderate.



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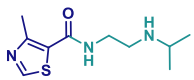
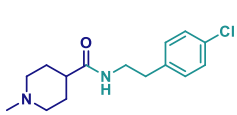
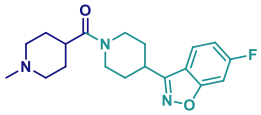


37%

Carboxylic acids bearing a trialkylated amine moiety showed much lower reactivity in the amide formation. Unreacted acid may not be fully scavenged during the purification step, which would remain in the final solution of the crude product.

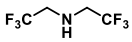


< 30%
inseparable mixture of product
and other compounds



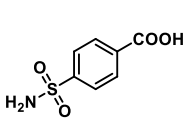
36%

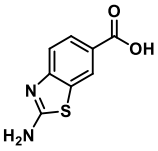
Additional list of identified compounds with low to no reactivity:

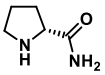


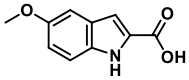
Solubility
Carboxylic acid and free alkyl amine need to be soluble in the reaction solvent of anhydrous EtOH and anhydrous CH_2Cl_2. Sonication may be used to help dissolving starting materials. Insoluble materials may cause damage to the synthesizer.

List of identified compounds with poor solubility in reaction solvents:









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

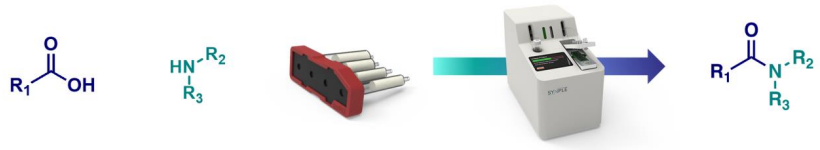
The purification step of the sequence can be disabled.

Reaction Planning

Solubility of reactants
Carboxylic acid and free alkyl amine shall be soluble in the reaction solvents (EtOH and CH_2Cl_2).

Tolerance of air and/or moisture
Amide formation 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 amine) gave comparable yields as the reactions without additional H_2O.

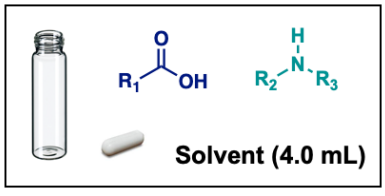
Sample Preparation



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

Setup
Components for sample preparation:

- Vial
- Amine (0.5 mmol) and carboxylic acid (0.55 mmol, 1.1 equiv)
- Stirbar
- Anhydrous EtOH (2.0 mL, 99.5%)
  Anhydrous CH_2Cl_2 (2.0 mL, 99.8%, amylene stabilized)
  Sonication may help dissolving insoluble materials.



Guide of selecting sequences

1) For primary amines without additional basic sites, select standard sequence (6 h). Reaction time can be shortened to 3 h (10800 s) if needed.
2) For primary amines with additional basic sites, select standard sequence (6 h).
3) For secondary amines, select standard sequence (6 h).
4) For sterically hindered amines, reaction time can be prolonged to 16 h (57600 s, see Reaction Parameter Editing).

Machine solvents for the use with Amide Formation 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 Reaction

1) Run automated MeOH wash after the amide formation reaction.
2) Run automated CH_2Cl_2 wash before starting a new amide formation 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