

## Application Note – Biotin tags (amide)

### Introduction

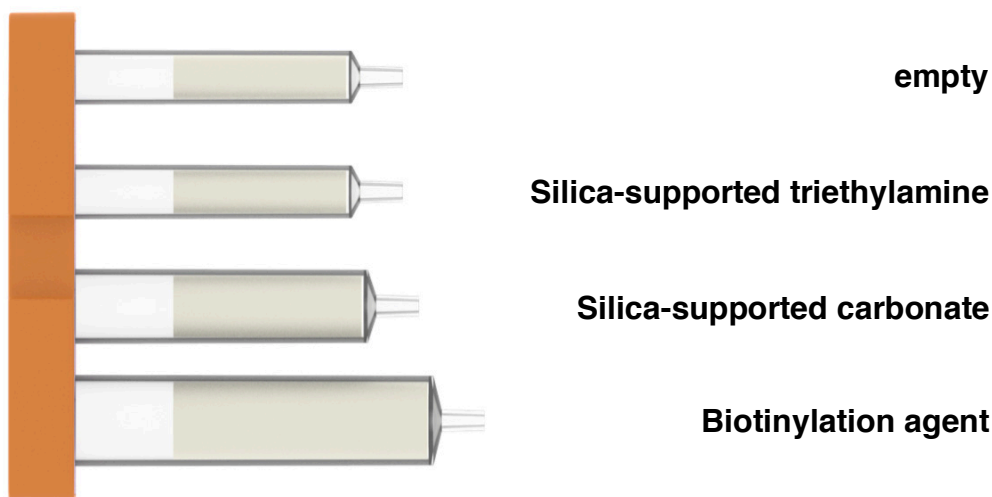
Biotinylation, also known as biotin labelling, is an important biochemical transformation of covalently linking biotin to a protein, nucleic acid or other molecules. The high affinity and specificity between biotin and streptavidin/avidin at a wide range of temperatures, pHs and solvents make biotinylation an attractive and reliable approach for protein detection, identification and purification. Biotinylated proteins can also be used to study protein-protein interaction and other research applications.

Using the approach described in this application note, the Synple Chem synthesizer offers an easy and fast automated method to prepare biotin tags (amide).



### Cartridge Contents

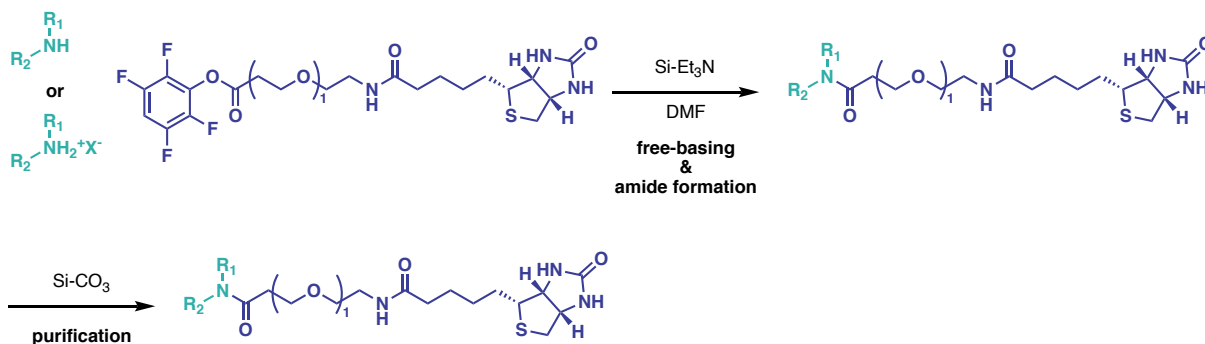
The cartridge contains a set of reagents to carry out a biotin amine forming reaction on a scale up to 0.1 mmol. Different linkers and linker lengths are available. Cartridge with biotinylation agents bearing a secondary or primary amine terminal end to couple to aldehydes and ketones are also available. Please refer to the application note – Biotin tags (amine)



### Reaction Scheme

This section describes the general course of the biotin tag (amide) formation by amide formation:

The cartridge contains the biotinylation agent in form of an active ester. In the first step free amine or amine salt undergoes free-basing and then added to the biotinylation agent. In the next step, the amide is formed followed by a purification on silica-supported carbonate.



## Reaction Procedure

### 1) Free basing

In the first step the solution of the free amine or amine salt in DMF was circulated through compartment 2 (free basing agent) to give the free amine. Compartment 2 is then rinsed into the vial with anhydrous  $CH_2Cl_2$ .

### 2) Amide Formation

In the next step the solution was circulated through compartment 4 (biotinylation agent) at 1 mL/min at room temperature for 0.5 h and then left in the vial for 3.5 h. When amide formation is complete, compartment 4 was rinsed with anhydrous  $CH_2Cl_2$  into the vial.

### 3) Purification & product release:

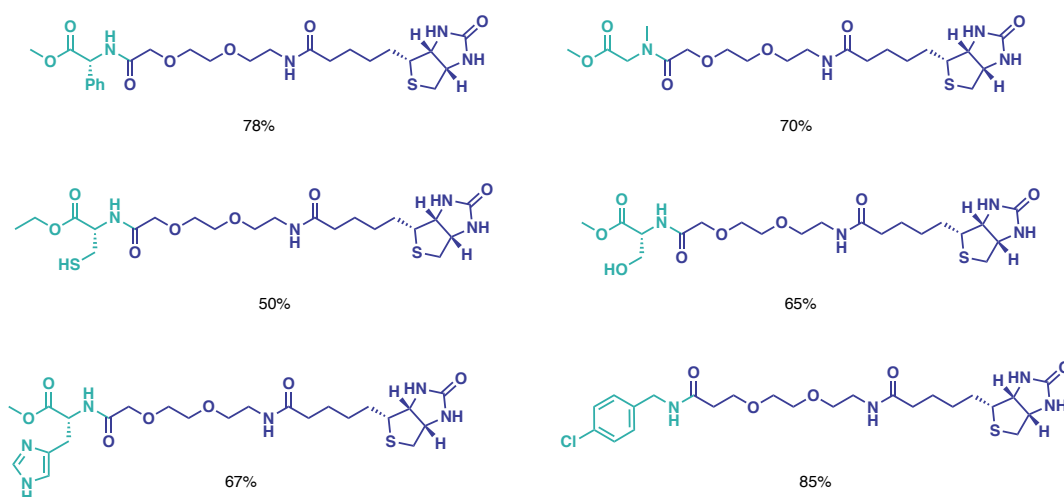
The reaction mixture was loaded to compartment 3 (acid scavenger) at 2 mL/min. Compartment 3 was further washed with anhydrous  $CH_2Cl_2$  and combined filtrate contained the biotinylated product.

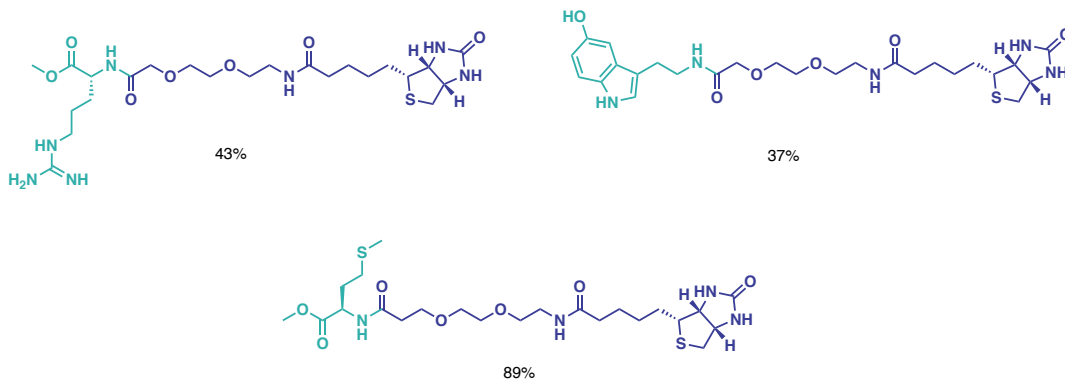
## Substrate Scope

### Tolerated functional groups

A wide range of different functional groups is tolerated. When the amine salt contains two equivalents of acid, one equivalent of  $Et_3N$  was added to dissolve the salt in DMF.

### Example substrate scope





## Known Chemistry-Limitations

### Acidic functional groups

If the amine starting material contains an acidic functional group such as carboxylic acid or tetrazole, the product may be trapped by the acid scavenger. This can be avoided by disabling the SCX purification step.

## Reaction Parameter Editing

### Editing parameters:

Parameter 1	Reaction time for amide coupling (seconds)
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### Enabling and Disabling parts:

#### Part 1:

#### Amine Freebasing

Initially the amine starting material will go through a freebasing step on solid supported triethylamine. In case the amine is already used as the free base and potentially unstable during freebasing conditions this step can be disabled.

#### Part 2:

#### Purification step

The purification step of the reaction sequence can be disabled. In case of very acid sensitive functional groups the purification might not be suitable. The machine will then provide the reaction product in solution in the reaction vial after the reaction step.

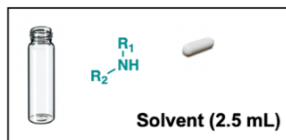
## Sample Preparation








**Setup**

Components for sample preparation:

- Vial
- Amine (up to 0.1 mmol)
- Stirbar
- 2.5 mL solvent (DMF)

**Machine Solvents for the use with Biotin tag cartridges**

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

	S1: Dichloromethane, anhydrous
	S2: –
	S3: –
	S4: –
	S5: –