Application Note – Biotin Tag Formation

(via Amide Formation)

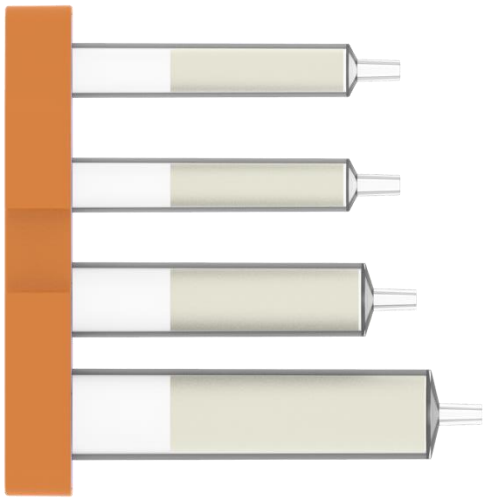
Introduction

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 via amide formation.

Cartridge Contents

The cartridge contains a set of reagents to synthesize biotin tags on a scale up to 0.1 mmol.



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silica-supported triethylamine

silica-supported carbonate

Biotinylating agent

The method can be used for the following transformations:

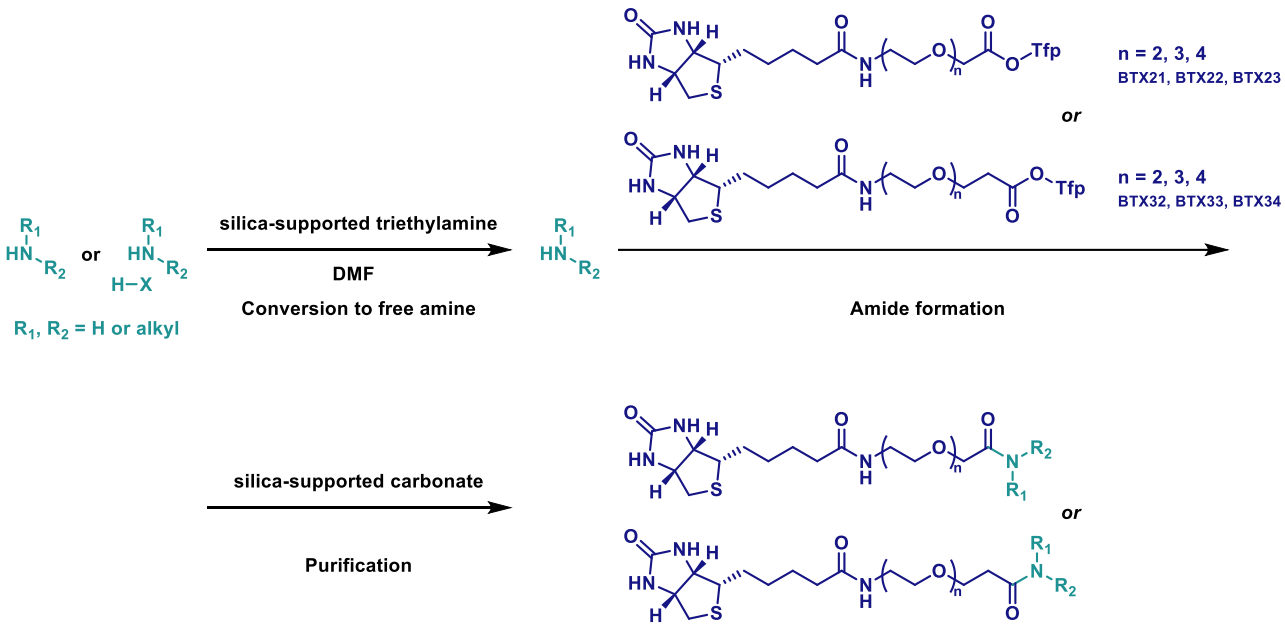
- Synthesis of biotin tags via amide formation between a biotinylating agent and a free alkyl amine or an alkyl amine salt.

Reaction Scheme

This section describes the general course of the biotin tag formation via amide formation:

The cartridge contains the biotinylating agent in form of an active ester. In the first step, the free alkyl amine or amine salt is converted into the free amine. Next, the free amine is mixed with the biotinylating agent to

undergo an amide formation to form the biotin tag. Upon completion, the crude mixture is purified over silica-supported carbonate.



Reaction Procedure

1) Conversion to free amine

In the first step, a solution of the free amine or amine salt in DMF is circulate through compartment 2 (silica-supported triethylamine) to provide the free amine. Compartment 2 is then rinsed with anhydrous CH_2Cl_2, which goes into the vial.

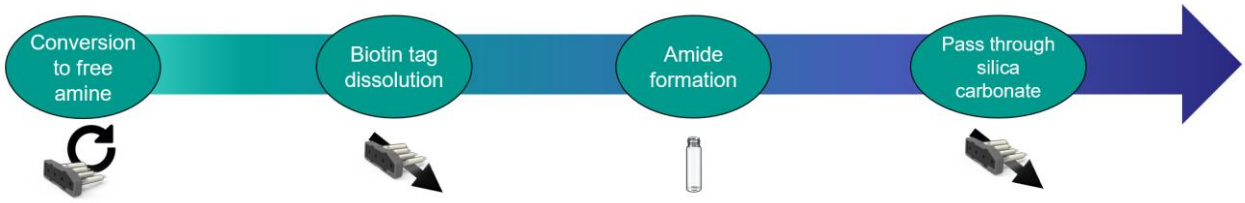
2) Amide formation

The solution is circulated through compartment 4 (biotinylating agent) at 1 mL/min for 0.5 h at room temperature and then left in the vial for 3.5 h. When the reaction is complete, compartment 4 is rinsed with anhydrous CH_2Cl_2, which goes into the vial.

3) Purification

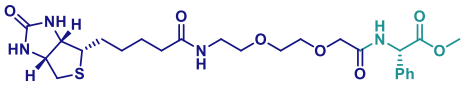
The reaction mixture is loaded to compartment 3 (silica-supported carbonate) at 2 mL/min. Compartment 3 is further rinsed with anhydrous CH_2Cl_2, which goes into the vial.

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



Substrate Scope

Example substrate scope



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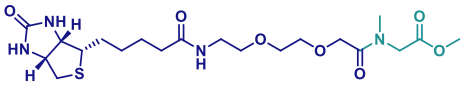

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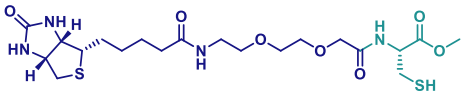

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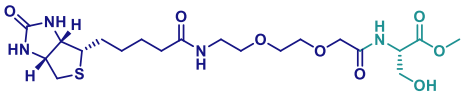

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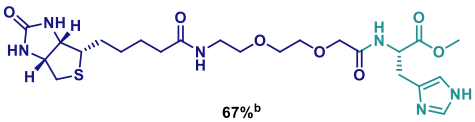

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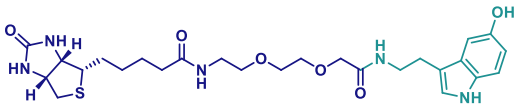

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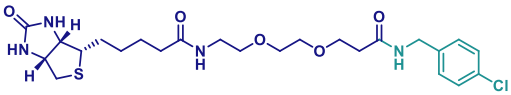

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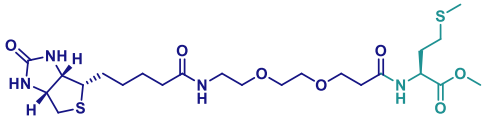

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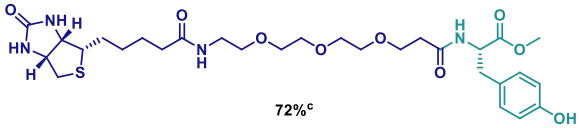

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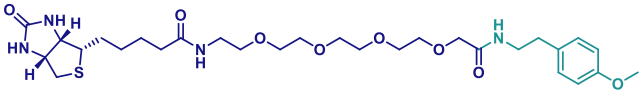

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a) from amine HCl salt.
b) from amine di-HCl salt.
c) from free amine.

Identified Chemistry Limitation

Reactivity
At present, the reaction has not been optimized for aryl amines and aryl amine salts.

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 silica-supported carbonate. This can be avoided by disabling the SCX purification step.

Product with high polarity
If the mass recovery is low, it is highly possible that the formed biotin tag product has high polarity, which is not fully eluted from compartment 3 (silica-supported carbonate). MeCN washing of compartment 3 will recover more product.

Reaction Parameter Editing

Editing parameters:

Parameter 1	Reaction time for amide coupling (seconds)

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.

Reaction Planning

Solubility
The amine or amine salt starting material shall be soluble initially in the reaction solvent (DMF).

Scale
The Synple automated biotin tag formation is suitable for a scale up to 0.1 mmol as the cartridge contains 0.1 mmol of the biotinylating agent.

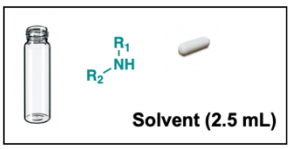
Tolerance of air and/or moisture
Biotin tag formation using Synple Chem synthesizer is insensitive toward air and moisture, although biotinylating agents are sensitive toward moisture. It is recommended that the cartridge shall be used directly after opening. Leaving the cartridge open for prolonged time may lead to hydrolysis of the biotinylating agent, therefore lower yield may be observed.

Sample Preparation

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

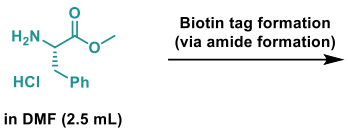
Setup
Components for sample preparation:

- Vial
- Alkyl amine or alkyl amine salt (0.1 mmol)
- Stirbar
- DMF (2.5 mL, ≥99%)

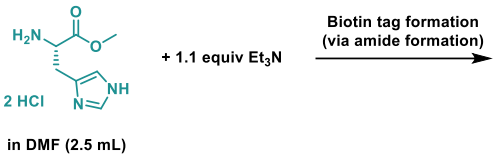


Guide of additives for sample preparation:

1) **Alkyl amines and alkyl amine mono salts.**
No additive.



2) **Alkyl amine di-salts.**
Et_3N (15 µL, 0.11 mmol, 1.1 equiv) is added.



Machine solvents for the use with Biotin Tag Formation (via 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: –
	S4: –
	S5: –

Machine Cleaning after Biotin Tag Formation (via Amide Formation)

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

Solvent Consumption and Run Time

SEQUENCE RUNTIME	
Reaction Sequence	**Time**
Biotin tag formation amine (via reductive amination)	13 h 47 min
Biotin tag formation amide (via amide formation)	4 h 36 min

SOLVENT COMSUMPTION FOR BIOTIN AMIDE	
For Reaction Setup	**Amount**
Dimethylformamide (DMF)	2.5 mL
Machine Solvents	
Dichloromethane (CH_2Cl_2)	20 mL
MeOH	20 mL