Application Note – Biotin Tag Formation

(via Reductive Amination)

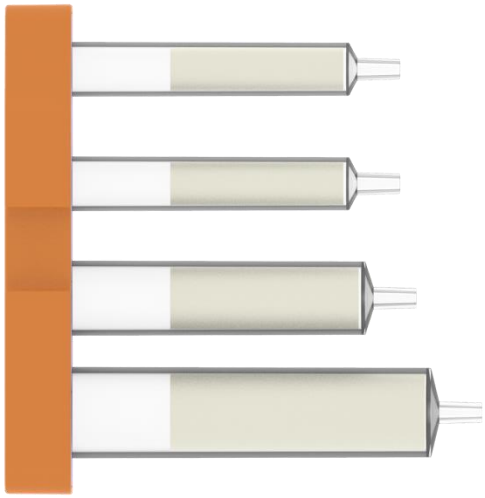
Introduction

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 reductive amination.

Cartridge Contents

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



silica-supported cyanoborohydride

silica-supported triethylamine

SCX

biotinylating agent

The method can be used for the following transformations:

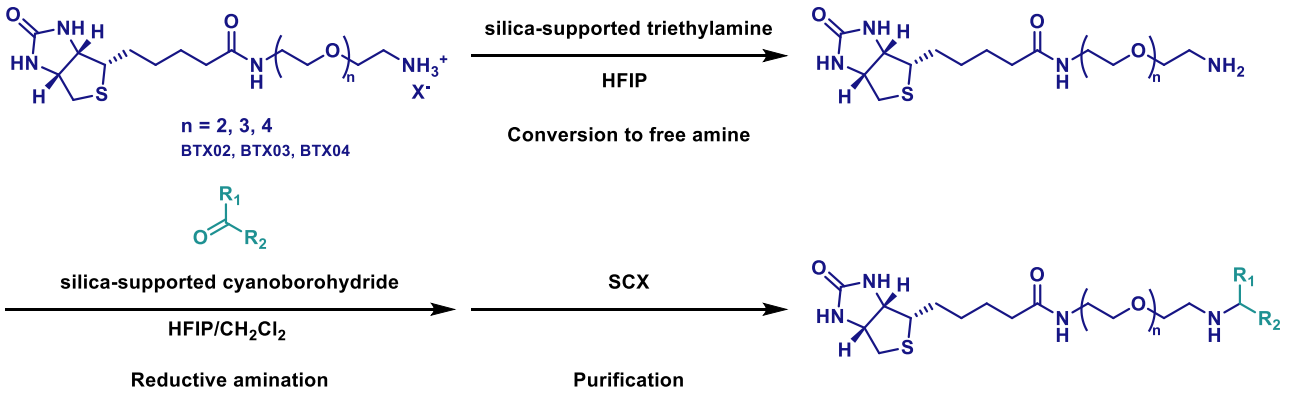
- Synthesis of biotin tags via reductive amination between a biotinylating agent and a carbonyl coupling partner (aldehyde or ketone).

Reaction Scheme

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

The cartridge contains the biotinylating agent in the form of an amine salt. In the first step the amine salt is converted to the free amine in the presence of the carbonyl coupling partner. Next, the free amine and the

carbonyl coupling partner undergo a reductive amination reaction to form the biotin tag. Upon completion, the crude mixture containing the product is purified using a catch & release strategy.



Reaction Procedure

1) Dissolution of biotinylating agent

In the first step, a solution of the carbonyl coupling partner in HFIP (1,1,1,3,3,3-hexafluoroisopropanol) is circulated through compartment 4 to dissolve the containing biotinylating agent. The compartment is then rinsed with anhydrous CH_2Cl_2, which goes into the vial.

2) Conversion to free amine

The solution containing the carbonyl coupling partner and the biotinylating agent is circulated through compartment 2 (silica-supported triethylamine) to convert the biotinylating agent from an amine salt into a free amine. Compartment 2 is further rinsed with anhydrous CH_2Cl_2, which goes into the vial.

3) Reductive amination

The solution containing the carbonyl coupling partner and biotinylating agent (as free amine) is circulated through compartment 1 (silica-supported cyanoborohydride) at 1 mL/min for 12 h at room temperature. When the reaction is complete, compartment 1 is rinsed with MeOH, which goes into the vial.

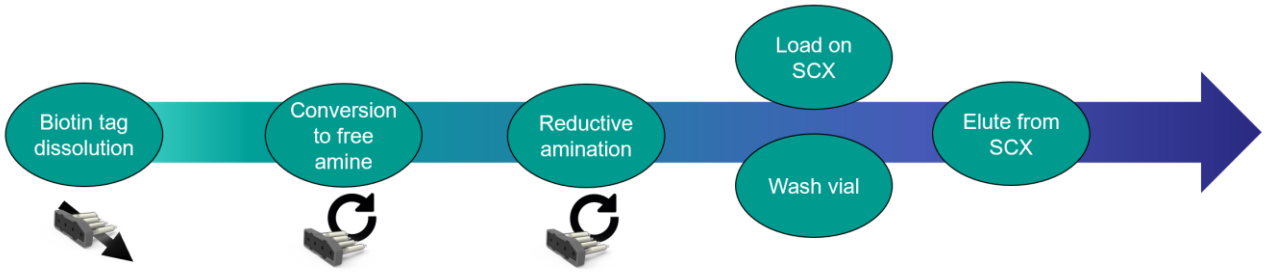
4) Purification

The reaction mixture is loaded to compartment 3 (SCX) at 2 mL/min. Compartment 3 is further rinsed with MeOH and CH_2Cl_2. The filtrate, which contains all the non-basic substances (formed biotin tag is basic and therefore trapped by SCX), is discarded to waste.

5) Product release

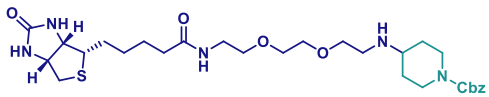
Compartment 3 is rinsed with 2.5 M *N*,*N*-diisopropylamine in MeOH (20 mL), which goes into the vial.

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



Substrate Scope

Example substrate scope



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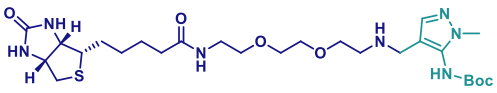

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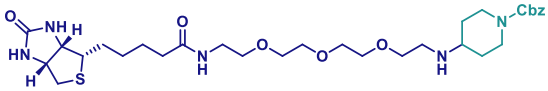

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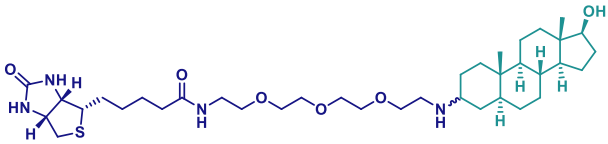

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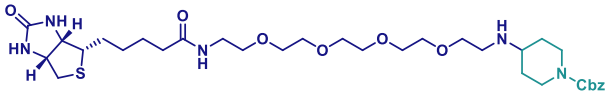

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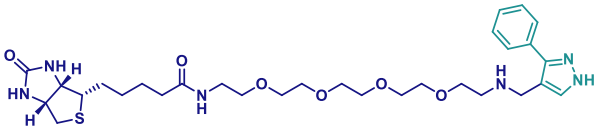

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Identified Chemistry Limitation

Synple automated biotin tag formation exploits reductive amination to generate biotin tags from carbonyl coupling partners. Therefore, some reported limitation in reductive amination reactions applies, for examples, reactions are sluggish with conjugated ketones. Sterically hindered ketones also react more slowly and may result in low conversions.

Basic functional groups
If the carbonyl starting material contains a basic functional group, an additional purification may be needed to remove the unreacted starting material and related side product, which are trapped and released together with the product.

Acid sensitive groups
In some cases, when Boc containing starting materials are used, certain amount of Boc deprotection can be observed. This can be avoided by disabling the SCX purification step.

Double reductive amination
When an aldehyde is used as the carbonyl coupling partner, double reductive amination may occur due to a secondary reaction between the formed biotin tag (secondary amine) and the aldehyde. In this case, an additional purification is necessary to obtain the pure biotin tag. To minimize this side reaction, it is suggested not to use an aldehyde in an excess amount.

Reaction Parameter Editing

Editing parameters:

Parameter 1	Reaction time for reduction (seconds)

Enabling and Disabling parts:

Part 1:
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 carbonyl starting material shall be soluble initially in the reaction solvent (HFIP).

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. When the carbonyl coupling partner is a ketone, excess amount can be used to improve both yield and purity of the crude biotin tag. When the carbonyl coupling partner is an aldehyde, it is recommended to use 1.0 equiv of the aldehyde (0.1 mmol) to minimize the side reaction of double reductive amination (see Identified Chemistry Limitation).

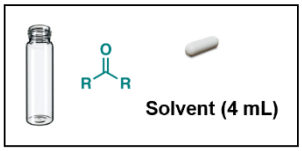
Tolerance of air and/or moisture
Biotin tag formation using Synple Chem synthesizer is insensitive toward air and moisture. As some biotinylating agents are highly hygroscopic, it is recommended that the cartridge shall be used directly after opening. Leaving the cartridge open for prolonged time may lead to lower yield and/or purity of the biotin tag product.

Sample Preparation

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

Setup
Components for sample preparation:

- Vial
- Aldehyde or ketone
- Stirbar
- HFIP (4.0 mL, 99.9%)



Guide of ratios for sample preparation

1) Aldehyde
   0.1 mmol (1.0 equiv) is added.

2) Ketone
   0.15 mmol (1.5 equiv) is added.

Machine solvents for the use with Biotin Tag Formation (via Reductive Amination) 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, >99.5%
	S4: *N*,*N*-diisopropylamine (175 mL, ≥99.8%) in MeOH (325 mL, ≥99.5%)
	S5: –

Machine Cleaning after Biotin Tag Formation (via Reductive Amination)

1) Run automated MeOH wash after the biotin tag formation reaction.

Solvent Consumption and Run Time

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

SOLVENT COMSUMPTION FOR BIOTIN AMINE	
For Reaction Setup	**Amount**
Hexafluoroisopropanol (HFIP) or Trifluoroethanol (TFE)	4 mL
Machine Solvents	
Dichloromethane (CH_2Cl_2)	74 mL
Methanol (MeOH)	74 mL
Diisopropylamine – MeOH mixture (13:7)	22 mL