Application Note – Biotin tags (amine)

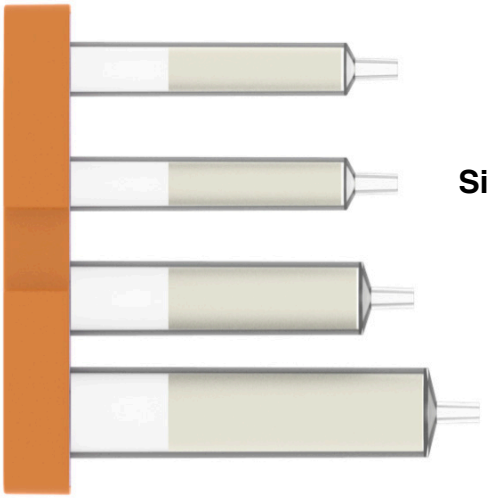
Introduction

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 (amine).

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 are available with primary and secondary amine terminal end. Cartridges forming an amide bond are also available; please refer to the application note – Biotin tags (amide).



Silica-supported cyanoborohydride

Silica-supported triethylamine or carbonate

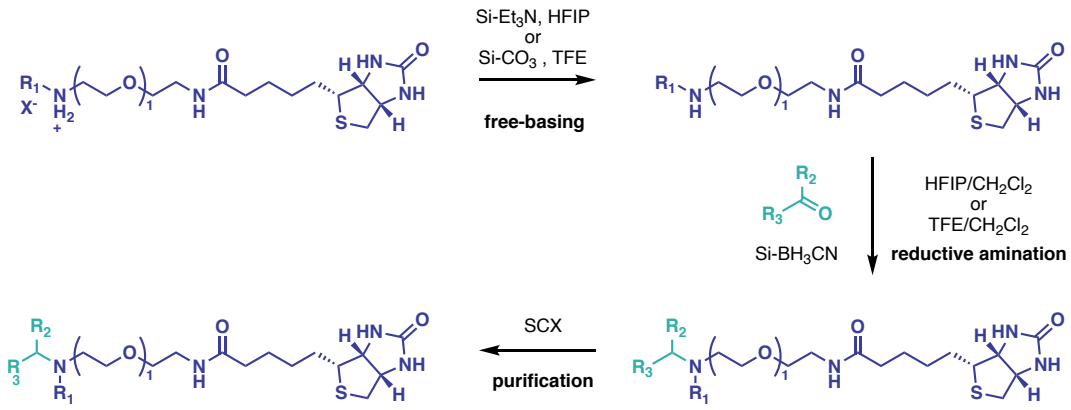
SCX

Biotinylation agent

Reaction Scheme

This section describes the general course of the biotin tag (amine) formation by reductive amination:

The cartridge contains the biotinylation agent in form of the amine salt. In the first step the amine salt undergoes free-basing and is then added to the carbonyl starting material. In the next step, the imine is reduced followed by a purification on SCX.



Reaction Procedure

1) Free-basing

In the first step the solution of the carbonyl compound in HFIP (1,1,1,3,3,3-hexafluoroisopropanol for 1° amine biotinylation agent) or TFE (2,2,2-trifluoroethanol for 2° amine biotinylation agent) was circulate through compartment 4 (biotinylation agent) to dissolve the biotinylation agent. The compartment is then rinsed into the vial with anhydrous CH_2Cl_2. The reaction liquid is then passed through compartment 2 (free basing agent) and then rinsed into the vial with anhydrous CH_2Cl_2.

2) Imine Formation / Reduction

In the next step the solution was circulated through compartment 1 (reducing agent) at 1 mL/min at room temperature for 12 h. When reduction is complete, compartment 1 was rinsed with MeOH into the vial.

3) Purification:

The reaction mixture was loaded to compartment 3 (SCX) at 2 mL/min. The compartment was then washed with MeOH and CH_2Cl_2.

4) Product release:

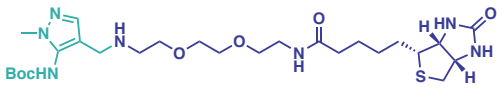
Compartment 3 was washed with 2.5 M DIPA/MeOH. The filtrate contained the biotinylated product.

Substrate Scope

Tolerated functional groups

A wide range of different functional groups is tolerated. At present, the reaction involving a secondary amine linker (piperidine) has not been fully optimized for sterically hindered aldehydes and ketones, which resulted in low yield of the product.

Example substrate scope



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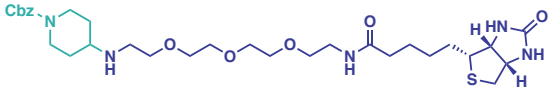

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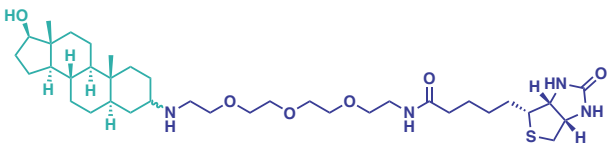

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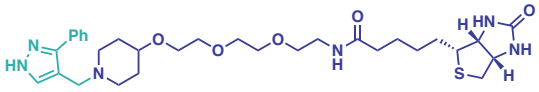

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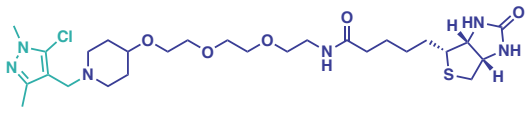

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Known Chemistry-Limitations

Low reactivity
Biotinylation agents incorporating the secondary amine linker showed low reactivity with ketones for the formation of tertiary amine product.

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. A new method of buffering SCX is under development, which avoids the cleavage of the Boc group.

Reaction Parameter Editing

Editing parameters:

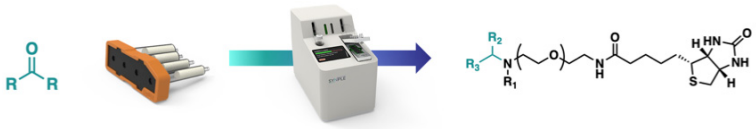
Parameter 1	Reaction time for reduction (seconds)

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.

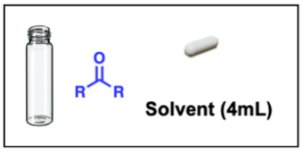
Sample Preparation



Setup

Components for sample preparation:

- Vial
- Aldehyde / Ketone (up to 0.1 mmol)
- Stirbar
- 4 mL solvent
  HFIP for primary biotinylation reagents
  TFE for secondary biotinylation reagents



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: MeOH
	S4: Diisopropylamine (175 mL) in MeOH (325 mL)
	S5: –