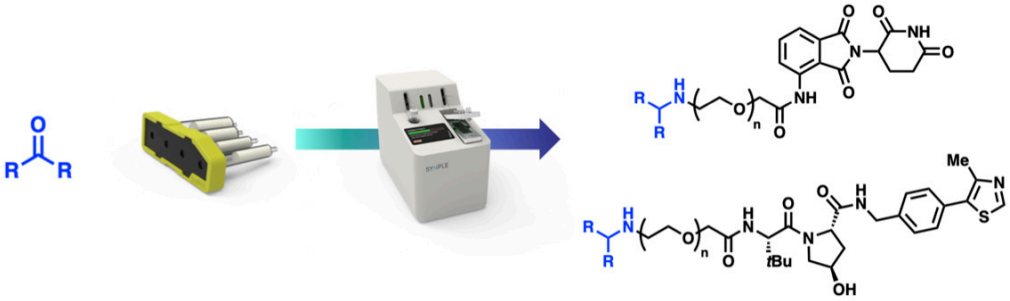
Application Note – PROTAC formation

(via reductive amination)

Introduction

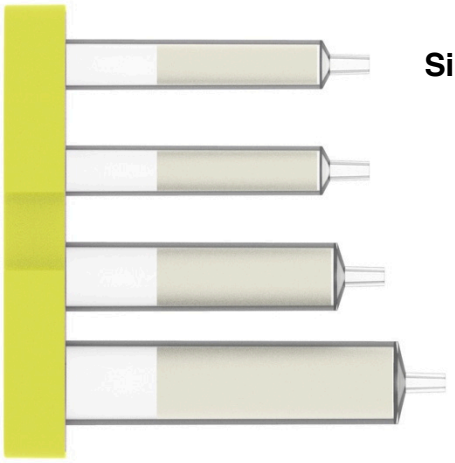
A proteolysis targeting chimera (PROTAC) is a bifunctional molecule, made up of a target protein binder linked to an E3 ubiquitin ligase binder, that is capable of selectively inducing the degradation of unwanted proteins. Originally described around the turn of the century, in the last five years PROTACs have become one of the hottest new fields in drug discovery.



The cartridges and methods provided with the Synple Chem synthesizer enable the user to rapidly prepare PROTAC analogues in an automated fashion and avoids the need for the manual manipulation of the difficult to handle partial PROTAC materials.

Cartridge Contents

The cartridge contains a set of reagents to form the PROTAC on a scale up to 0.1 mmol via a reductive amination with an aldehyde or ketone.



Silica supported cyano borohydride

Solid supported triethylamine

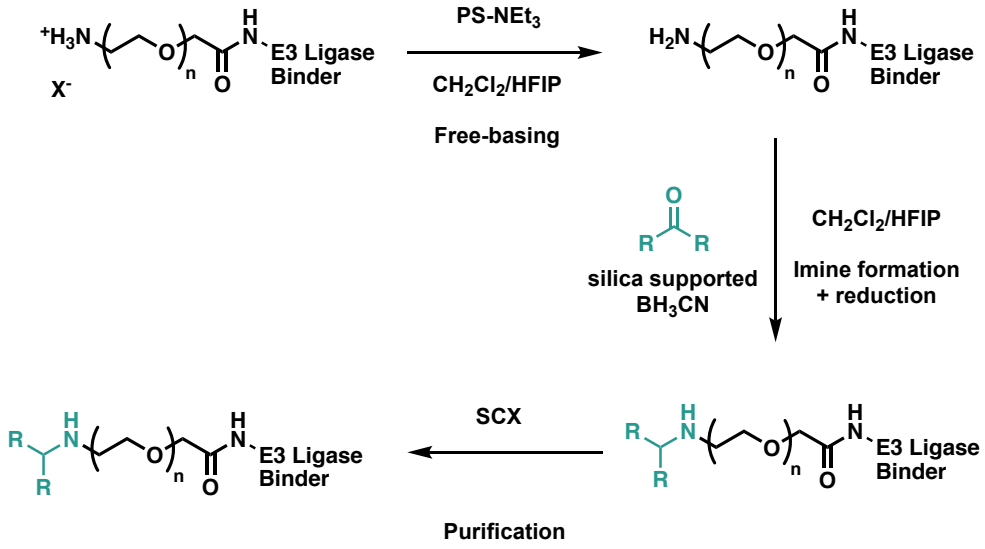
SCX

Partial PROTAC reagent

Reaction Scheme

This section describes the general course of the PROTAC formation by reductive amination:

The cartridge contains the partial PROTAC 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 is compartment 4 (PROTAC) to dissolve the partial PROTAC. 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

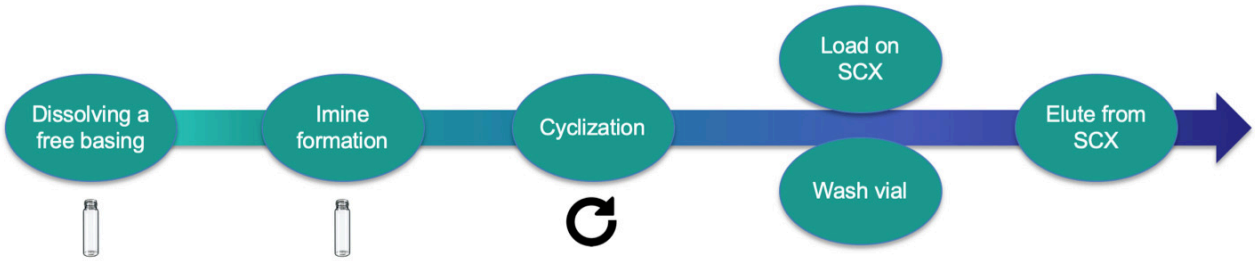
In the next step the solution was circulated through cartridge compartment 1 (reducing agent) at 2 mL/min at room temperature for 2.5 h. After the imine formation is complete, compartment 1 was rinsed with CH_2Cl_2 (5 mL) into the vial.

3) Purification:

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

4) Product release:

Compartment 3 was washed with 2.5 M DIPA/IPA (15mL). The filtrate contained the PROTAC product.



Substrate Scope

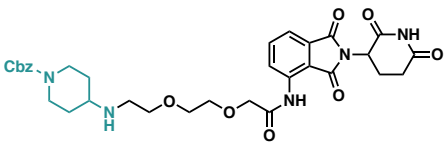
Tolerated functional groups

A large amount of different functional groups are tolerated.

At present the reaction has not been fully optimized for aliphatic aldehydes. The reaction does work but the purity is less than ideal so efforts are on-going to optimize these.

Example substrate scope

CRBN-PROTACs



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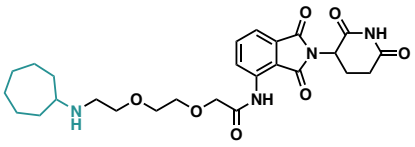

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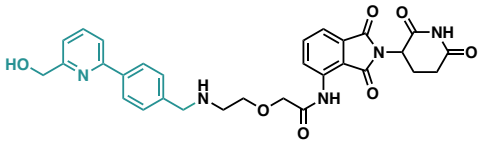

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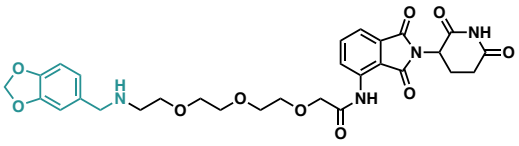

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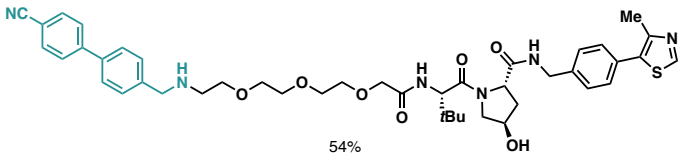
VHL-PROTACs



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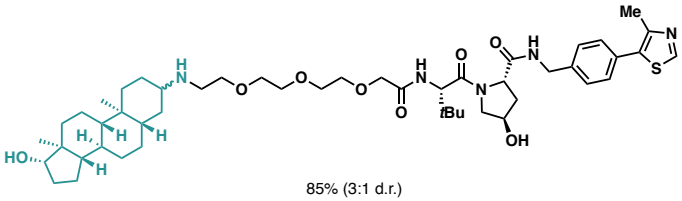

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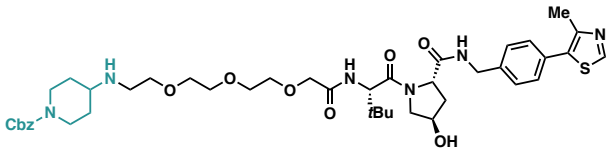

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Known Chemistry-Limitations

Insoluble starting materials
The aldehyde and ketones must be soluble in when the sample is prepared initially. Insoluble materials will lead to no or low conversion, in the worst case, lead to blockage of the system.

Basic functional groups
If the aldehyde/ketone contains a basic group a further purification step may be needed, because any excess starting material and side products might contaminate the product.

Boc deprotection
For rare cases when Boc containing starting materials are employed, a certain extent of Boc deprotection can be observed. This can be avoided by disabling the SCX purification step.

Stability of CRBN ligand

The CRBN ligand is by its nature not too stable. The half-life in MeOH at 40°C is roughly 30 min by opening of the imide. This often leads to a second visible peak when injected into an HPLC / LCMS, which runs on MeOH.

Reaction Parameter Editing

Editing parameters:

Parameter 1	Reaction time for reduction (seconds)
Parameter 2	Amount of solvent for elution from “catch & release” resin: In case of very polar substrates more solvent could be required to wash of the last bit of product from the catch & release resin. Therefore, the value can be increase. To calculate the input value multiply the volume in mL by 600. For example the value 9000 is equivalent to 15 mL (Maximum value 12000)

Enabling and Disabling parts:

Part 1:
Purification step

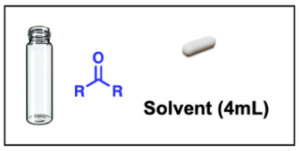
The purification step of the sequence can be disabled. In case of very acid sensitive functional groups the purification might not be suitable. The machine will then provide the reaction product in solution in the reaction vial after the reduction step.

Sample Preparation

Setup

Components for sample preparation:

- Vial
- Aldehyde / Ketone (up to 0.1 mmol)
- Stirbar
- 4 mL solvent (CH_2Cl_2 : HFIP = 3:1 (4 mL)



Machine Solvents for the use with PROTAC cartridges

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

	S1: Dichloromethane, anhydrous
	S2: –
	S3: MeOH
	S4: Diispropylamine (175 mL) in Isopropanol (325 mL)
	S5: –