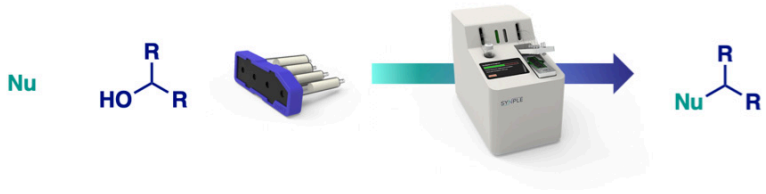
Application Note – Mitsunobu

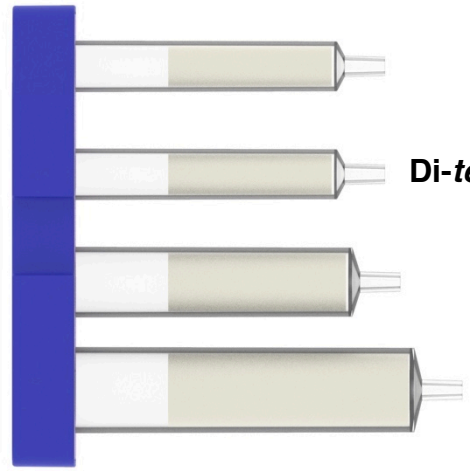
Introduction

Since its discovery in 1967, Mitsunobu reaction has got a privileged role in organic synthesis and medicinal chemistry because of its scope, stereoselectivity and mild reaction conditions. Its ability of easily forming carbon-carbon bond through dehydrative coupling of a primary or secondary alcohol with a pronucleophile (generally phenols or carboxylic acids, but also imides, sulfonamides, oximes, hydrazides…) is sometimes counterbalanced by the formation of byproducts which can plague purification strategies.
Using the approach described in this application note, the Synple Chem synthesizer offers an easy and fast automated method for carrying out Mitsunobu reaction overcoming all the purification related issues.



Cartridge Contents

The cartridge contains a set of reagents to carry out a Mitsunobu reaction on a scale up to 0.5 mmol.



Triphenylphosphine
(polymer supported)

Di-*tert*-butyl Azodicarboxylate DTBAD

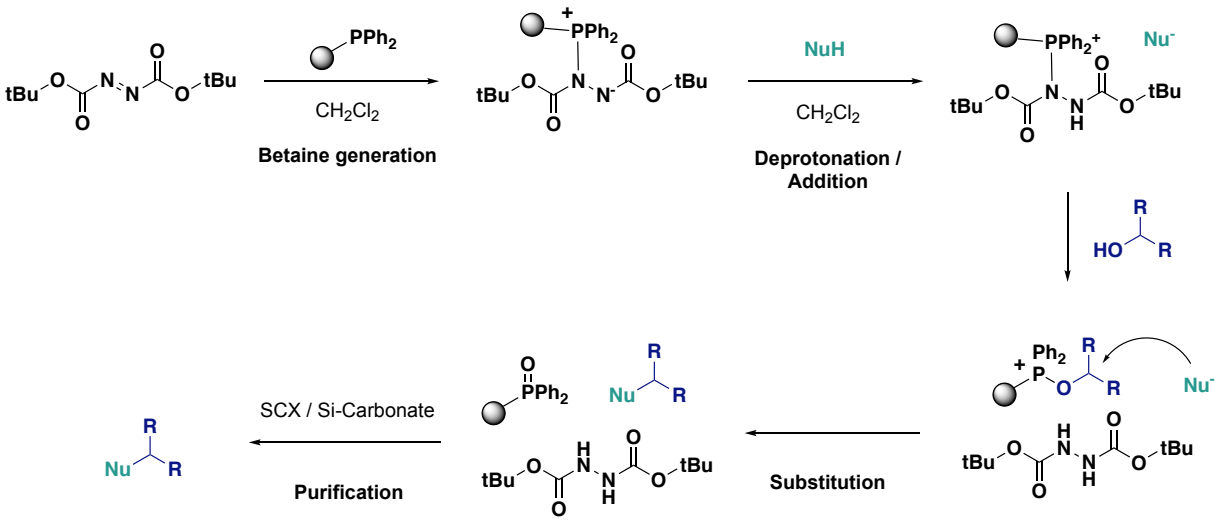
SCX

Silica Supported Carbonate
(Acid Scavenger)

Reaction Scheme

This section describes the general course of the Mitsunobu reaction:

Initially the polymer supported triphenylphosphine makes a nucleophilic attack on the azodicarboxylate species to generate the betaine. The nucleophile is then deprotonated by the betaine and the alcohol forms the oxyphosphonium ion with the betaine intermediate. The deprotonated nucleophile makes a substitution on the phosphorous intermediate to form the desired Mitsunobu reaction product. The formed phosphinoxide specie stays on the solid support and does not contaminate the crude. After the reaction the formed hydrazine by-product is destroyed by treatment with the supported sulfonic acid SCX, giving at the end a crude without the presence of the usual two major contaminants present in the classical batch reaction.



Reaction Procedure

Reaction sequences:
For carrying out the reaction on the machine two sequences are available for selection. The standard **Mitsunobu** should be run except for substrates that contain basic moieties (N-Heterocycles, amines). For such substrates the "**Mitsunobu basic**" sequence should be selected. Detailed steps of the reaction on the machine are outlined below.

Detailed Overview over Mitsunobu and Mitsunobu basic reaction sequences:

MITSUNOBU:

1) **Phosphine swelling and reagents dissolution**

In the first step, CH_2Cl_2 (3 mL) was loaded into compartment 1 (polymer supported triphenylphosphine) to swell the resin, and then to the vial, to dissolve the starting materials. The mixture was stirred for 2 minutes to make sure all the starting materials are dissolved. 3 mL of CH_2Cl_2 were then loaded into compartment 2 (di-tert-butyl azodicarboxylate – DTBAD) to dissolve the reagent and add it to the vial. The solution was circulated for 1 minute through compartment 2 to dissolve all the reagent, then the compartment was washed with 2 mL of CH_2Cl_2.

2) **Reaction**

The solution was circulated through compartment 1 for 4 hours at 3 mL/min at room temperature. After the reaction is complete, the resin in compartment 1 was washed with 4 mL of CH_2Cl_2.

3) **SCX purification**

The reaction mixture was circulated through compartment 3 (SCX) for 2 hours to remove the hydrazine byproduct of DTBAD. The compartment was then washed with 6 mL of CH_2Cl_2.

4) **Nucleophile removal**

The solution was loaded into compartment 4 (Silica supported carbonate) to remove any excess of phenol or carboxylic acid in the reaction crude. The compartment was then washed with 4 mL of CH_2Cl_2.

MITSUNOBU BASIC:

1) **Phosphine swelling and reagents dissolution**

Same as Mitsunobu sequence

2) Reaction

Same as Mitsunobu sequence

3) Nucleophile removal

The solution was loaded into compartment 4 (Silica supported carbonate) to remove any excess of phenol or carboxylic acid in the reaction crude. The compartment was then washed with 4 mL of CH_2Cl_2.

4) SCX purification

The solution was loaded into compartment 3 (SCX). The non-basic substances (DTBAD-hydrazine, excess starting material) were removed washing the resin with 5 mL of CH_2Cl_2 and 15 mL of 2-propanol. The product was released from the acidic resin by washing with 15 mL of DIPA (2.5 M solution in 2-propanol)

Substrate Scope

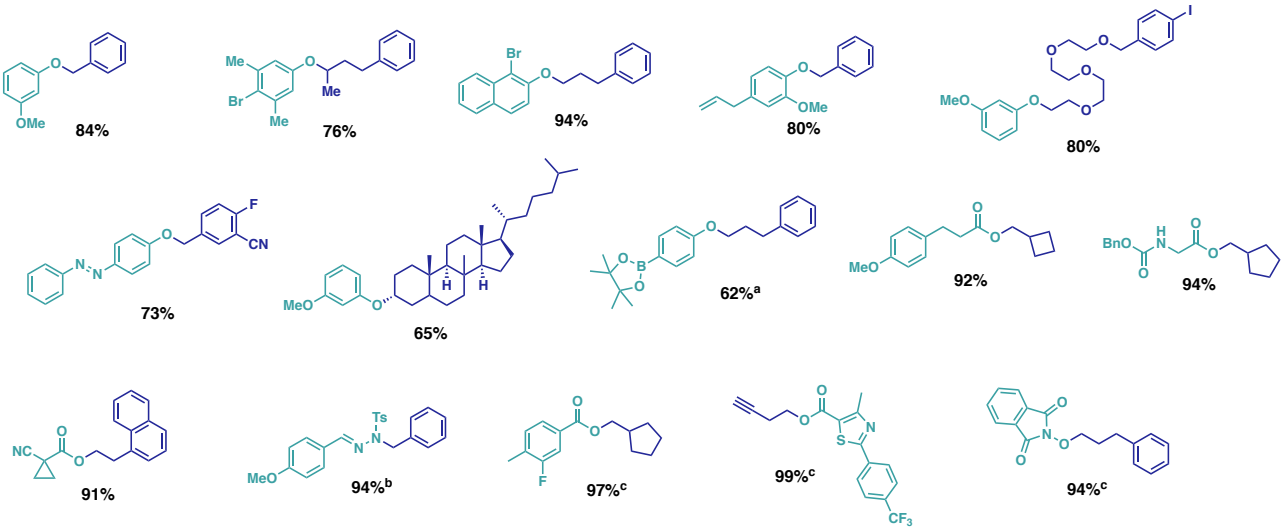
Supported alcohols

Primary and secondary alcohols are supported.

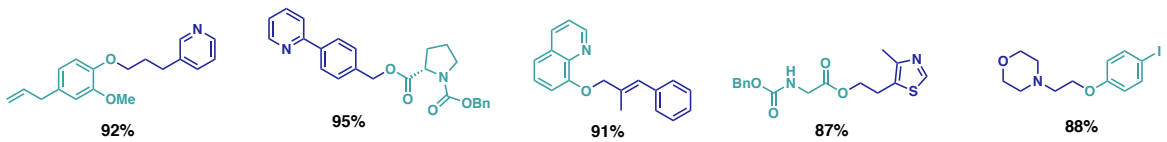
Supported nucleophiles

Phenols, carboxylic acids, tosyl amides and hydrazones are supported in the reaction.

Example substrate scope



"Basic Mitsunobu" sequence:



a) SCX-2 purification disabled, isolated yield after flash chromatography
b) Isolated yield after flash chromatography
c) Starting material insoluble in DCM: dry THF bottle used in place of dry DCM (see below: "Known chemistry limitations —> Insoluble starting materials") . Isolated yield after flash chromatography

Known Chemistry-Limitations

The Synple automated Mitsunobu suffers from all the known limitations of the Mitsunobu chemistry. Apart from that, the following points should be taken into consideration. A solution (if applicable) is also provided.

Insoluble starting materials
The reaction performs better if both starting materials are soluble in CH_2Cl_2. Insoluble materials will lead to no or low conversion or, in the worst case, lead to blockage of the system. Starting materials can be dissolved in 1 ml of dry THF, dry MeCN or dry DMF prior connection to the machine. A slight drop in reaction/purification performance can be observed. If dry DMF is used the SCX purification of the standard Mitsunobu sequence will not work properly, and further purification must be performed to remove the DTBAD hydrazine byproduct. In exceptional cases, the CH_2Cl_2 bottle can be replaced with dry THF. A drop in reaction/purification performance can be observed and further purification may be required. Is recommended to put back the CH_2Cl_2 bottle after the sequence is complete.

Acid sensitive groups
Compounds containing acid sensitive moieties, such as Boc, silyl ethers or acetal protecting groups, may be not stable in the purification step due to the acidity of the SCX. The work-up and purification can be disabled for such compounds.

Other nucleophiles
The carbonate purification step is able to remove excess phenol or carboxylic acid from the crude. Any other kind of nucleophiles (i.e. tosyl amides) cannot be removed in this step and further purifications will be required afterwards.

Known impurities
Commonly observed: undegraded hydrazine byproduct (<5%), excess nucleophile not completely removed (<5%), alcohol starting material if reaction is incomplete, DIPA and/or 2-propanol (only on Mitsunobu Basic, can be stripped away with CH_2Cl_2 or chloroform).
Less common: isopropyl ester or isopropyl aryl ether of the starting nucleophile (<5%, may be due to missing/insufficient washing of the system before the reaction. Check if the washing solvent - CH_2Cl_2 - ran out during the washing sequence)

Reaction Parameter Editing

Mitsunobu sequence:

Editing parameters:

Parameter 1	Reaction time (seconds)

Enabling and Disabling parts:

Part 1:
Purification step including acid scavenging

The purification step of the sequence can be disabled, which also disable the removal the hydrazine byproduct of DTBAD. 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 completed reaction step.

Mitsunobu basic sequence

Editing parameters:

Parameter 1	Reaction time (seconds)

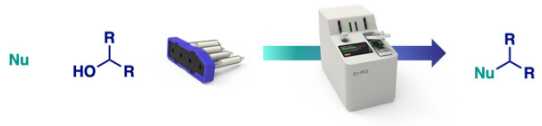
Parameter 2	Amount of solvent for elution from “catch & release” resin: In case of very polar substrates more solvent could be required to wash of the last bit of product from the catch & release resin. Therefore, the value can be increase. To calculate the input value multiply the volume in mL by 600. 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 completed reaction step.

Sample Preparation



Setup

Components for sample preparation:

- Vial
- Alcohol 1.0 equiv. / Nucleophile 1.5 equiv.
- Stir bar
- No solvent/1 ml dry CH_2Cl_2

Starting materials can be put in the vial neat or dissolved in 1 ml of dry CH_2Cl_2 (or dry THF/MeCN/DMF, see **known chemistry/limitations** section). Reaction performs optimally on 0.5 mmol of alcohol, with 0.75 mmol (1.5 equiv.) of nucleophile. Positive outcome is guaranteed with lesser amount of alcohol, down to 0.2 mmol. For Mitsunobu Basic, ideal conditions that would yield clean product require the reagent with the basic group as the limiting reagent.

Machine Solvents for the use with Mitsunobu cartridges

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

	S1: Dichloromethane, anhydrous
	S2: –
	S3: 2-propanol *only needed for basic sequence
	S4: Diispropylamine (175 mL) in 2-propanol (325 mL) *only needed for basic sequence
	S5: –