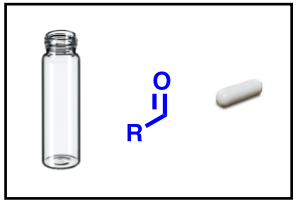
Reaction sample preparation – N-Heterocycle formation

Version: 10.01.2022

Setup



Sample Setup:

- Vial
- Aldehyde
- Stir bar
- No solvent

Machine Solvents

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

Amount Aldehyde: 0-0.5 mmol

Solvent: Neat

Requirements: aldehyde soluble in CH_2Cl_2

Variants: Full sequence (11 hours) for highest conversion

Short sequence (5 hours) to obtain sample or for electron poor aldehydes

Reaction Parameter Editing

Editable Parameters:

- Parameter 1: Imine formation temperature of cartridge (°C)
- Parameter 2: Imine formation temperature of reaction vial (°C)
- Parameter 3: Imine formation reaction time (seconds)
- Parameter 4: Cyclization temperature of cartridge (°C)
- Parameter 5: Cyclization temperature of reaction vial (°C)
- Parameter 6: Cyclization reaction time (seconds)

For more information see Application Note – N-Heterocycle

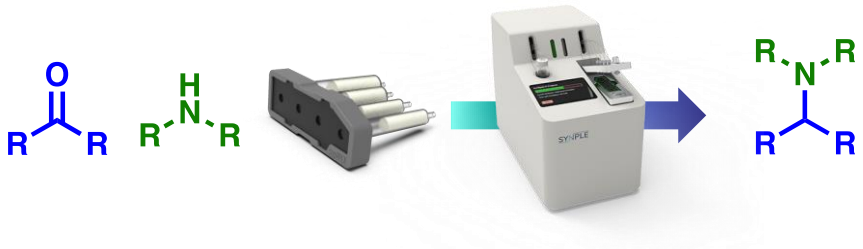
Editable Parts:

- Part 1: Substrate solvation step
- Part 2: Purification step

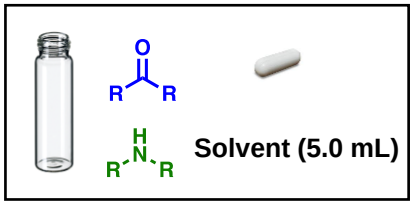
Additional Notes: 1) Check solvent level before setup. 2) aldehydes containing beta-heteroatom are not supported (catalyst complexation). 3) Run “Wash DCM” Sequence between each run.

Reaction sample preparation – Reductive Amination

Version: 10.01.2022



Setup



Sample Setup:
- Vial
- Aldehyde / Ketone
- Amine
- Stir bar
- 5 mL solvent (see variants)

Machine Solvents

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

Variants

Aldehyde + 1° Amine

Aldehyde + 2° Amine

Ketone + 1° Amine

Substrate ratios for best result:

Aldehyde : 1° Amine	Aldehyde : 2° Amine	Ketone : 1° Amine
1:2	2:1	1:1 (to 2:1)

Amount: 0-0.5 mmol
Solvent: CH_2Cl_2 : HFIP = 4:1 (5 mL) for best result
CH_2Cl_2 can be partially replaced with MeOH for solubility

Ketone + 2° Amine

Substrate ratio for best result: Ketone : 2° Amine = 2:1
Amount: 0-0.5 mmol
Solvent: Toluene (5 mL) – can be replaced with MeOH for solubility

Reaction Parameter Editing

Editable Parameters:

Ketones + 2° Amines:
- Parameter 1: Reduction step temperature cartridge
- Parameter 2: Reduction step temperature vial
- Parameter 3: Reaction time for reduction (sec.)
- Parameter 4: Amount of solvent for elution of product from SCX

Other sequences:
- Parameter 1: Reaction time for reduction (seconds)
- Parameter 2: Amount of solvent for elution of product from SCX

Editable Parts:

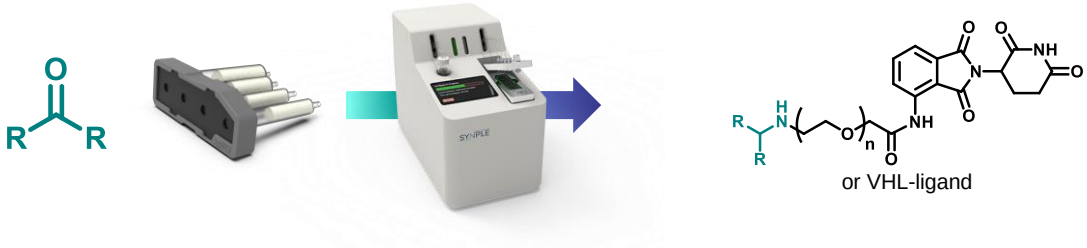
Aldehydes + 1° Amines
- Part 1: Excess amine scavenging
- Part 2: Purification step

Other sequences:
- Part 1: Purification step

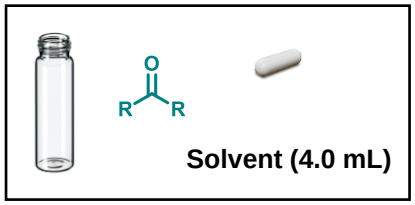
Additional Notes: 1) Check solvent level before setup. 2) Run Wash DCM Sequence between each run.

Reaction sample preparation – PROTAC (amine)

Version: 10.01.2022



Setup



Sample Setup:
- Vial
- Aldehyde / Ketone
- Stir bar
- 4 mL solvent (see right)

Machine Solvents

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

Amount Aldehyde/Ketone: 0-0.1 mmol

Solvent: CH_2Cl_2 : HFIP = 3:1 (4 mL)

Requirements: aldehyde/ketone soluble in solvent mixture

Reaction time: 13 hours

For Cartridges: P001-P025 and P101-P125
P001L-P025L and P101L-P125L

Reaction Parameter Editing

Editable Parameters:

- Parameter 1: Reaction time of reduction (seconds)
- Parameter 2: Amount of solvent for elution of product from SCX

Editable Parts:

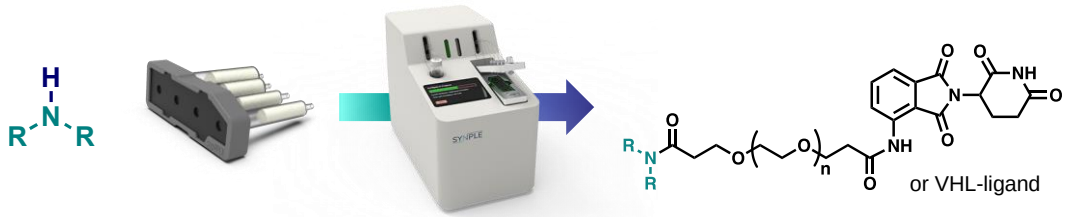
- Part 1: Purification step

For more information see Application Note – PROTAC (amine)

Additional Notes: 1) Avoid handling the CRBN products in protic solvents, due to instability of the Pomalidomide core ($t_{1/2}$ ~30 min in MeOH, 40˚C). 2) Check solvent level before setup. 3) Run Wash DCM Sequence between each run.

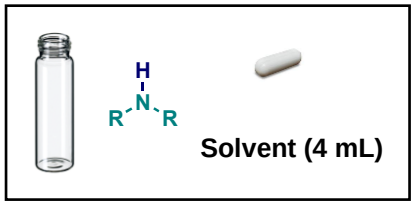
Reaction sample preparation – PROTAC (active ester)

Version: 10.01.2022



Discontinued – use PROTAC via amide formation as replacement (P060-P085)

Setup



Sample Setup:
- Vial
- Primary or secondary amine
- Stir bar
- 4 mL solvent (see right)

Machine Solvents

- S1: Dichloromethane, anhydrous
- S2: –
- S3: Isopropanol
- S4: –
- S5: –

Amount Aldehyde/Ketone:	0-0.1 mmol
Solvent:	4 mL of preferably ACN, DMF, EtOAc, CH_2Cl_2
Requirements:	amine soluble in chosen solvent
Variants:	currently only free amine; amine salts possible soon
For Cartridges:	P040-P065 and P140-P165

Reaction Parameter Editing

Editable Parameters:

- Parameter 1: Reaction time of amide formation

For more information see Application Note – PROTAC (amide)

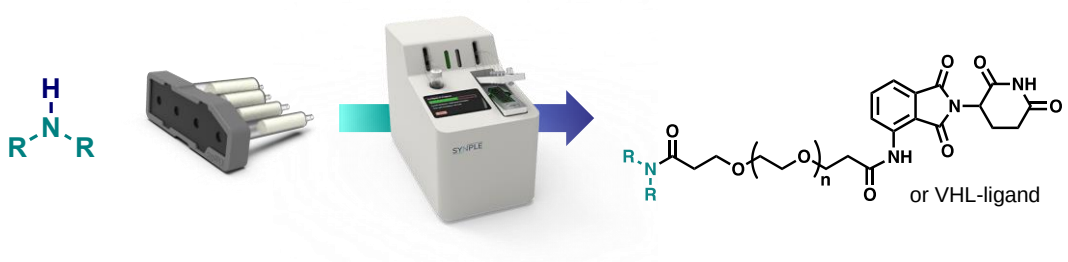
Editable Parts:

- Part 1: Purification step

Additional Notes: 1) Avoid handling the CRBN products in protic solvents, due to instability of the Pomalidomide core ($t_{1/2}$ ~30 min in MeOH, 40˚C). 2) Check solvent level before setup. 3) Run Wash DCM Sequence between each run.

Reaction sample preparation – PROTAC (amide)

Version: 10.01.2022



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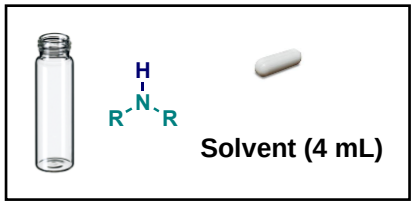


Sample Setup:

- Vial
- Primary or secondary amine
- Stir bar
- 4 mL solvent (see right)

Machine Solvents

- S1: Dichloromethane, anhydrous
- S2: –
- S3: Isopropanol
- S4: –
- S5: –

Amount Aldehyde/Ketone: 0-0.1 mmol

Solvent: 2 mL DCM + 2mL MeCN

Requirements: amine soluble in chosen solvent

Variants: currently only free amine; amine salts possible soon

For Cartridges: P060-P085 and P160-P185

Reaction Parameter Editing

Editable Parameters:

- Parameter 1: Reaction time of amide formation

For more information see Application Note – PROTAC (amide)

Editable Parts:

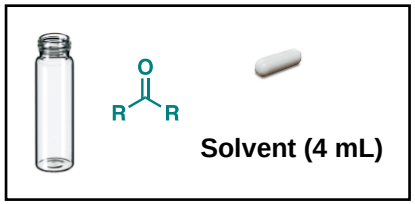
- Part 1: Purification step

Additional Notes: 1) Avoid handling the CRBN products in protic solvents to long, due to instability of the Pomalidomide core ($t_{1/2}$ ~30 min in MeOH, 40˚C). 2) Check solvent level before setup. 3) Run Wash DCM Sequence between each run.

Reaction sample preparation – Biotin synthesis (amine)

Version: 10.01.2022

Setup



Sample Setup:

- Vial
- Aldehyde / Ketone
- Stir bar
- 4 mL solvent (see right)

Machine Solvents

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

Amount Aldehyde/Ketone: 0-0.1 mmol

Solvent: HFIP (4 mL) for primary biotinylation reagents
TFE (4 mL) for secondary biotinylation reagents

Requirements: aldehyde/ketone soluble in solvent mixture

Reaction time: 15 hours

Reaction Parameter Editing

Editable Parameters:

- Parameter 1: Reaction time for reduction (seconds)

For more information see Application Note – Biotin (amine)

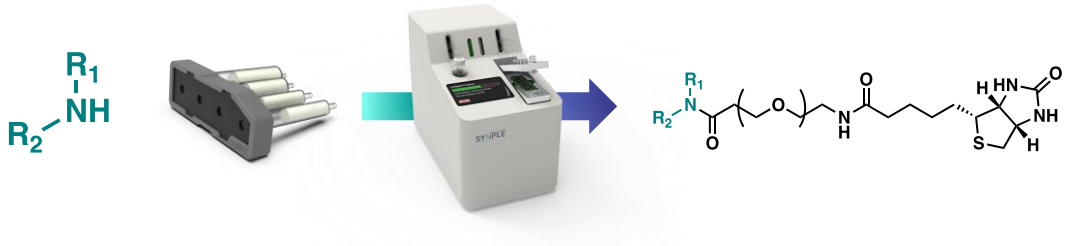
Editable Parts:

- Part 1: Purification step

Additional Notes: 1) Check solvent level before setup. 2) Run Wash MeOH Sequence between each run to remove insoluble Biotin residues.

Reaction sample preparation – Biotin synthesis (amide)

Version: 10.01.2022



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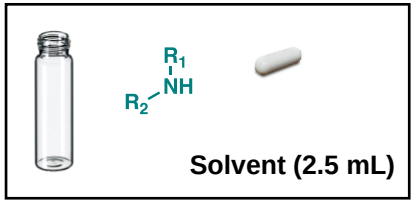


Sample Setup:

- Vial
- Amine or amine salt
- Stir bar
- 2.5 mL solvent (see right)

Machine Solvents

- S1: Dichloromethane, anhydrous
- S2: –
- S3: MeOH
- S4: –
- S5: –

Amount Aldehyde/Ketone: 0-0.1 mmol

Solvent: DMF (2.5 mL)

Requirements: amine soluble in DMF

Reaction time: 6 hours

Reaction Parameter Editing

Editable Parameters:

- Parameter 1: Reaction time for amide formation (seconds)

For more information see Application Note – Biotin (amide)

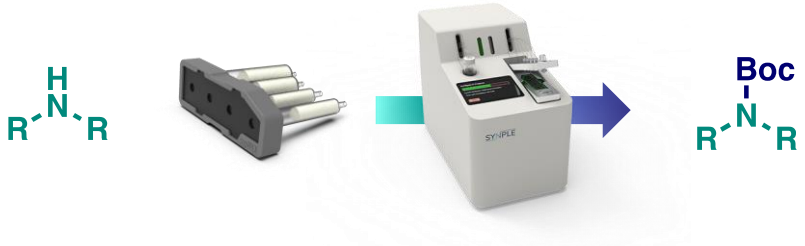
Editable Parts:

- Part 1: Amine Freebasing
- Part 2: Purification step

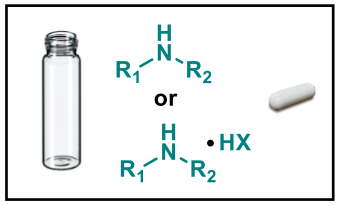
Additional Notes: 1) Check solvent level before setup. 2) Run Wash MeOH Sequence between each run to remove insoluble Biotin residues.

Reaction sample preparation – N-Boc protection

Version: 10.01.2022



Setup



Sample Setup:

- Vial
- Amine
- Stir bar
- No solvent

Machine Solvents

- S1: Dichloromethane, anhydrous
- S2: –
- S3: MeOH
- S4: –
- S5: –

Amount Amine: 0-0.5 mmol for B001
0-1.2 mmol for B002

Solvent: Neat

Requirements: amine MeOH soluble

Sequence: Choose between “free amine” and “amine salt” depending on the state of the amine substrate

Reaction Parameter Editing

Editable Parameters:	Editable Parts:
• Parameter 1: Reaction time for Boc protection (seconds)	–

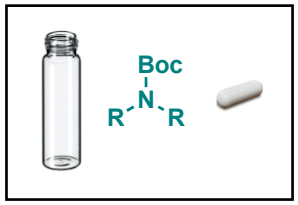
For more information see Application Note – Boc protection

Additional Notes: 1) Check solvent level before setup. 2) Run Wash DCM or MeOH Sequence between each run.

Reaction sample preparation – N-Boc deprotection

Version: 10.01.2022

Setup



Sample Setup:

- Vial
- N-Boc starting material
- Stir bar
- No solvent

Machine Solvents

- S1: Dichloromethane, anhydrous
- S2: Dimethoxyethane (DME)
- S3: MeOH
- S4: –
- S5: –

Amount Amine: 0-0.5 mmol

Solvent: Neat

Requirements: substrates soluble in Dimethoxyethane (DME)

Reaction Parameter Editing

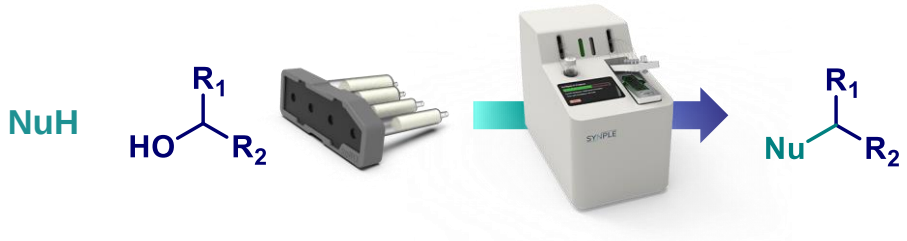
Editable Parameters:	Editable Parts:
• Parameter 1: Reaction time for Boc protection (seconds)	–

For more information see Application Note – Boc deprotection

Additional Notes: 1) Check solvent level before setup. 2) Run Wash DCM or MeOH Sequence between each run.

Reaction sample preparation – Mitsunobu

Version: 10.01.2022



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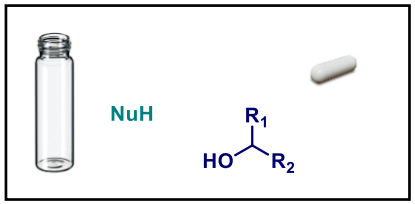


Sample Setup:

- Vial
- Alcohol and Nucleophile
- Stir bar
- No solvent

Machine Solvents

- S1: Dichloromethane, anhydrous
- S2: –
- S3: Ethanol
- S4: Diisopropylamine (175 mL) in Ethanol (325 mL)
- S5: –

Amount Substrates:	0.2 - 0.5 mmol
Substrate ratio:	1.0 equiv. alcohol / 1.5 equiv. nucleophile
Solvent:	Neat
	(THF can be added for solubility)
Requirements:	starting materials soluble in CH_2Cl_2
Reaction time:	6.5 hours
Sequence:	standard Mitsunobu
	Mitsunobu (basic substrates)

Current limitations and incompatibilities:

- Acid sensitive substrates not supported
- Substrates not soluble in CH_2Cl_2
- Yield may decreases up to 25% if less than 0.5 mmol of s.m. are used
- Not acidic nucleophiles (i.e. amides) cannot be removed by the purification system

Reaction Parameter Editing

Editable Parameters:

- Parameter 1: Reaction time (seconds)
- Parameter 2: Amount of solvent for elution of product from SCX

Editable Parts:

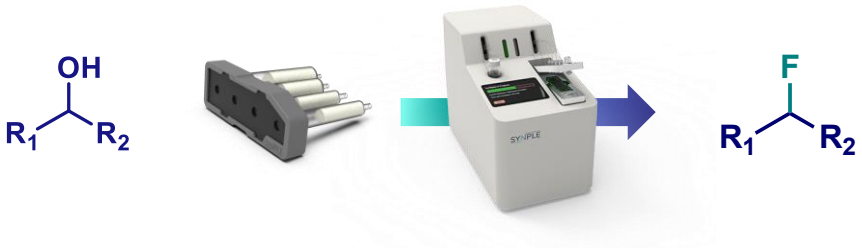
- Part 1: Purification step

For more information see Application Note – Mitsunobu

Additional Notes: 1) Check solvent level before setup. 2) Run Wash DCM Sequence between each run. If MeOH Wash Sequence was run before the reaction, some MeOH Addition product may be observed

Reaction sample preparation – Deoxyfluorination

Version: 10.01.2022



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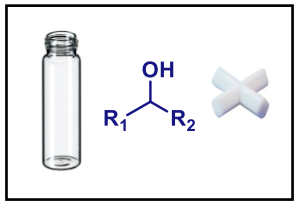


Sample Setup:

- Vial
- Alcohol starting material
- Crossed stir bar (if available)
- No solvent

Amount Alcohol: 0-0.2 mmol

Solvent: Neat

Machine Solvents

- S1: Dichloromethane, anhydrous
- S2: Toluene, anhydrous with molecular sieves (4A)
- S3: MeOH
- S4: –
- S5: Acetonitrile, HPLC grade

Reaction Parameter Editing

Editable Parameters:

- Parameter 1: Reaction time for Fluorination (seconds)

For more information see Application Note – Fluorination

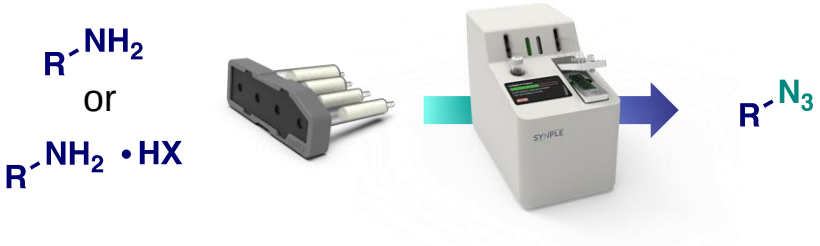
Editable Parts:

- Part 1: Purification

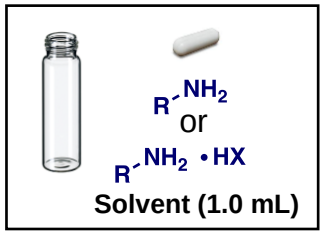
Additional Notes: 1) Check solvent level before setup. 2) Run Wash DCM Sequence between each run. If MeOH wash was used, a DCM wash is recommended before the fluorination reaction, since residual MeOH could interfere with the reaction.

Reaction sample preparation – Azide Formation

Version: 10.01.2022



Setup



Sample Setup:

- Vial
- Primary Amine or primary amine salt
- Stir bar
- 1 mL solvent (see right side)
- KHCO3 (see right side)

Machine Solvents

- S1: –
- S2: –
- S3: MeOH
- S4: –
- S5: Acetonitrile : H_2O = 1 : 1

Amount Alcohol: 0-0.5 mmol

Solvent:

For primary amines:	Acetonitrile : H_2O = 1:1 (1.0 mL)
For primary amine salts:	Acetonitrile : H_2O = 1:1 (1.0 mL) + equimolar amount of solid $KHCO_3$ to HX

Requirements: Soluble in Acetonitrile : H_2O mixture

Reaction Parameter Editing

Editable Parameters:

- Parameter 1: Reaction time for Azide Formation (seconds)

For more information see Application Note – Azide Formation

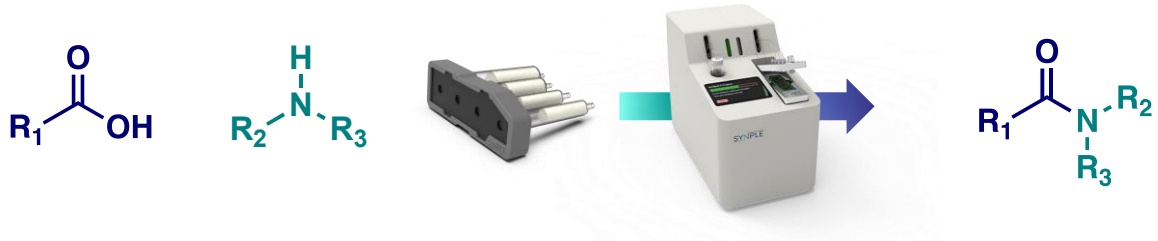
Editable Parts:

- Part 1: Purification

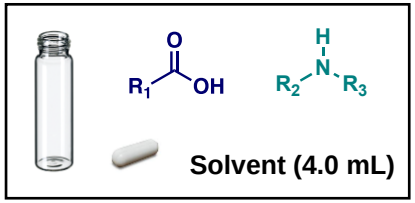
Additional Notes: 1) Check solvent level before setup. 2) Run MeOH Wash Sequence between each run. 3) When using materials containing basic functional groups (e.g. trialkylamines, imidazoles, pyridines, anilines) the purification step needs to be disabled to avoid the product being scavenged.

Reaction sample preparation – Amide formation

Version: 10.01.2022



Setup



Sample Setup:

- Vial
- Amine
- Carboxylic acid
- Stir bar
- 4.0 mL solvent (see right)

Machine Solvents

- S1: Dichloromethane, anhydrous
- S2: –
- S3: MeOH
- S4: –
- S5: –

Amount Amine: 0-0.5 mmol
Ratio Amine : Acid 1 : 1.1

Solvent: 4.0 mL (2.0 mL anhydrous EtOH + 2.0 mL anhydrous CH_2Cl_2)

Requirements: substrates soluble in EtOH + CH_2Cl_2 mixture

Reaction time: 6 hours for secondary amines. Can be shortened to 3 hours for primary amines (see application note for examples

Reaction Parameter Editing

Editable Parameters:

- Parameter 1: Reaction time for amide formation (seconds)

For more information see Application Note – Amide formation

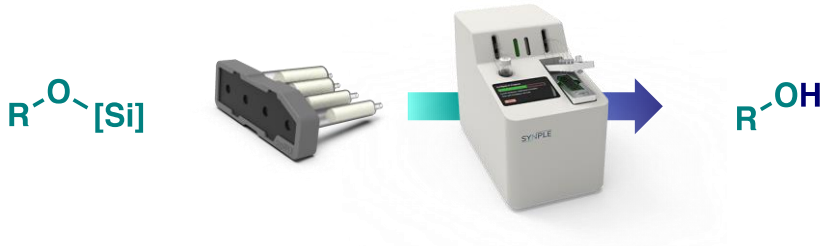
Editable Parts:

- Part 1: Purification step

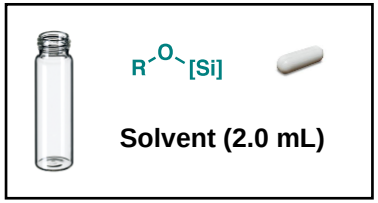
Additional Notes: 1) Check solvent level before setup. 2) Run MeOH wash sequence after reaction.

Reaction sample preparation – Silyl deprotection

Version: 10.01.2022



Setup



Sample Setup:
- Vial
- Silyl protected compound
- Stir bar
- 2.0 mL solvent (see right)

Machine Solvents

- S1: Dichloromethane, anhydrous
- S2: –
- S3: MeOH
- S4: –
- S5: –

Amount Substrate: 0-0.5 mmol
[Si| = TBS, TES, TIPS

Solvent:
- 2.0 mL MeOH for best result
- alternatively 2.0 mL EtOH or *i*PrOH (requires longer reaction time, e.g. 13 hours).
- in case of poor solubility a mixture of 1.0 mL CH_2Cl_2 + 1.0 mL MeOH can be used

Requirements: substrates soluble in the solvent mixture

Reaction Parameter Editing

Editable Parameters:

- Parameter 1: Reaction time for deprotection reaction (seconds)

For more information see Application Note – Silyl deprotection

Editable Parts:

- Part 1: Purification step

Additional Notes: 1) Check solvent level before setup. 2) Run MeOH wash sequence between each run.