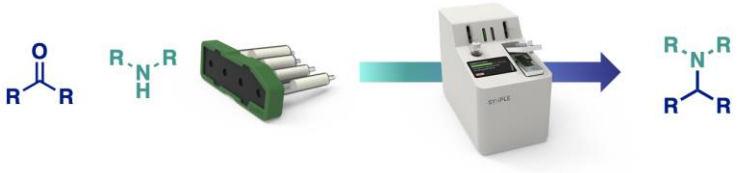
Application Note – Reductive Amination

Introduction

Reductive amination involves the formation of a new carbon-nitrogen bond via the reaction of a carbonyl compound and an amine. This reaction is one of the most widely utilized reactions in medicinal chemistry, since the resulting amine products are ubiquitous among biologically active compounds. In a standard reductive amination, the imine/iminium intermediate formed by condensation between an amine and a carbonyl compound is reduced to amine by the reducing agents. Commonly employed reducing agents for reductive aminations are $NaBH_3CN$ or $NaBH(OAc)_3$. In some cases, the imine/iminium intermediate is preformed prior to the addition of the reducing agent; and in these cases, besides the two hydride reductants mentioned above, then the reduction can also be achieved with $NaBH_4$ or H_2 with Pd/C.

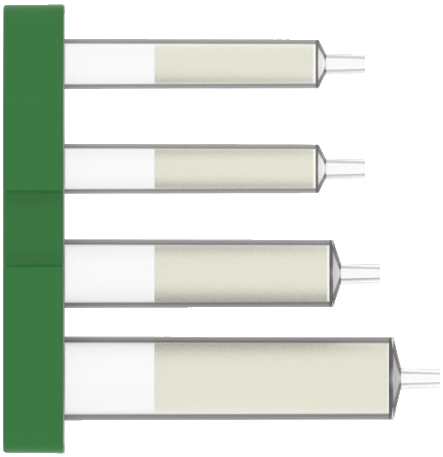
Silica-supported cyanoborohydride was identified as the reducing agent of choice for the Synple automated reductive amination. Combining this solid-supported reductant with solid-phase extraction strategy, reductive amination reaction has become more user-friendly requiring neither manual workup nor time-consuming purification.



Using the approach described in this application note, the Synple Chem synthesizer offers an easy and fast automated method for the reductive amination reaction between amines and carbonyl compounds.

Cartridge Contents

The cartridge contains a set of reagents to carry out the reductive amination on a scale of up to 0.5 mmol.



silica-supported cyanoborohydride

phenylacetic acid

SCX

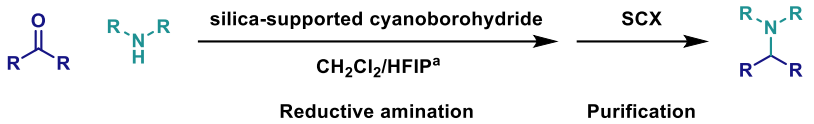
polymer-supported benzaldehyde

The method can be used for the following transformations:

- Reductive amination between an aldehyde and a primary amine.
- Reductive amination between an aldehyde and a secondary amine.
- Reductive amination between a ketone and a primary amine.
- Reductive amination between a ketone and a secondary amine.

Reaction Scheme

This section describes the general course of the reductive amination:



a) HFIP = 1,1,1,3,3,3-hexafluoropropanol

References and Publications:

(1) Afanasyev, O. I.; Kuchuk, E.; Usanov, D. L.; Chusov, D. Reductive Amination in the Synthesis of Pharmaceuticals. *Chem. Rev.* **2019**, *119* (23), 11857–11911. Link.

(2) Tripathi, R.; Verma, S.; Pandey, J.; Tiwari, V. Recent Development on Catalytic Reductive Amination and Applications. *Curr. Org. Chem.* **2008**, *12* (13), 1093–1115. Link.

Reaction Procedure

1) Reductive amination

In the first step, the solution of the amine and the aldehyde (or ketone) in CH_2Cl_2 and HFIP (1,1,1,3,3,3-hexafluoroisopropanol) is circulated through cartridge compartment 1 (silica-supported cyanoborohydride) at 2 mL/min at room temperature for the designated time (see table below). After reductve amination is complete, compartment 1 is rinsed with anhydrous CH_2Cl_2 (4.0 mL), which goes into the vial.

Reductive Amination Type	Time of reductive amination	Time of full sequence
Aldehyde + primary amine	3 h	4 h 45 min
Aldehyde + secondary amine	3 h	4 h 45 min
Ketone + primary amine	3 h	4 h 25 min
Ketone + secondary amine	4 h	5 h 30 min

NOTE: For *ketone* + *secondary amine*, the reaction mixture is passed through compartment 2 (phenylacetic acid) before starting the reductive amination reaction. Compartment 2 is then rinsed with anhydrous CH_2Cl_2 (2.0 mL), which goes into the vial.

2) Excess amine scavenger (only in *aldehydes + primary amine*)

The reaction mixture is passed through compartment 4 (polymer-supported benzaldehyde) at 1.0 mL/min. Unreacted or excess amount primary amine is scavenged in this step. Compartment 4 is then rinsed with anhydrous CH_2Cl_2, which goes into the vial.

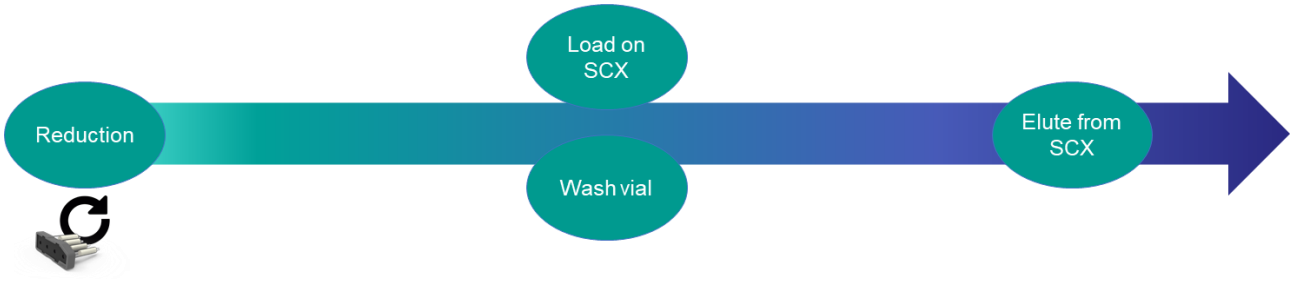
3) SCX purification

The solution in the vial is loaded into compartment 3 (SCX) at 2.0 mL/min. The compartment is then rinsed with MeOH and anhydrous CH_2Cl_2.

4) Product release

Compartment 3 (SCX) is rinsed with 15 mL of 2.5 M *N,N*-diisopropylamine in MeOH

After product release, the solution in the vial contains the reductive amination product.



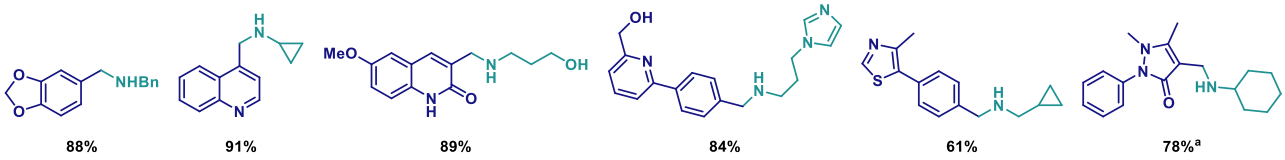
Substrate Scope

Tolerated functional groups
Alcohols, phenols, esters, carboxylic acids, alkenes, alkynes, nitriles, nitro groups, protected amines (Cbz, Ac, etc.), and various heterocycles are fully tolerated. Acetals, *tert*-butoxycarbonyl (Boc) and other acid sensitive functional groups are also tolerated, but partial cleavage can be observed (see Identified Chemistry Limitation).

***N*-Methylation**
N-Methylation of an amine via reductive amination can be achieved using 2 equivalents of formaldehyde (30% aqueous solution), though a certain amount of cyanomethylated product may be formed (see Identified Chemistry Limitation).

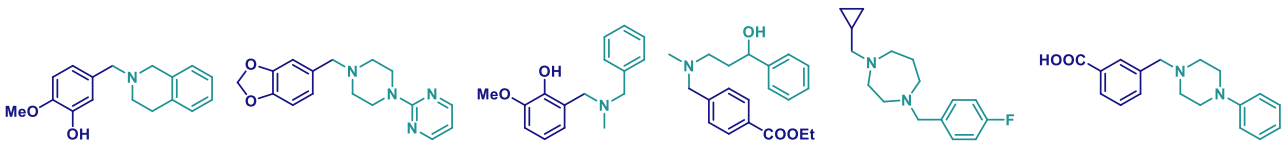
Example substrate scope

Aldehyde + primary amine (from 0.5 mmol aldehyde)



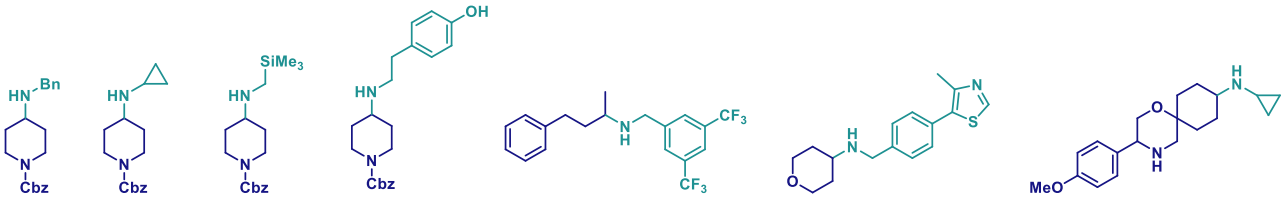
88% 91% 89% 84% 61% 78%[a]

Aldehyde + secondary amine (from 0.5 mmol amine)



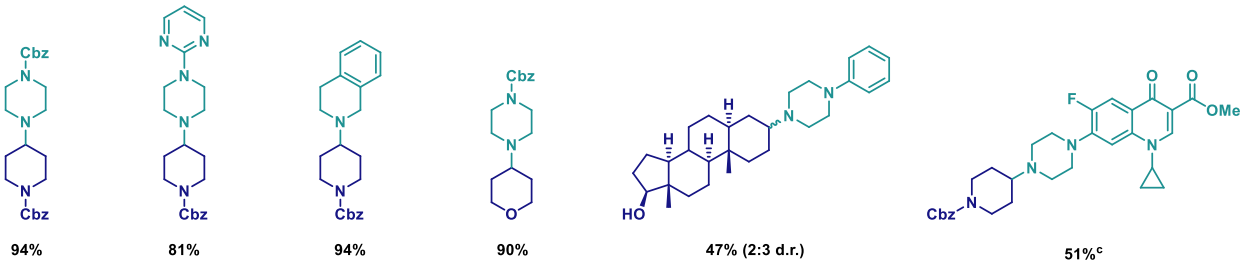
99% 86% 100% 61% 84% 82%[b]

Ketone + primary amine (from 0.5 mmol amine)



89% 96% 92% 78% 80% 61% 91%

Ketone + secondary amine (from 0.5 mmol amine)



94% 81% 94% 90% 47% (2:3 d.r.) 51%[c]

a) Reaction time set to 5 h.
b) Product obtained as diisopropylamine carboxylate salt.
c) Incomplete reaction.Purified by flash chromatography.

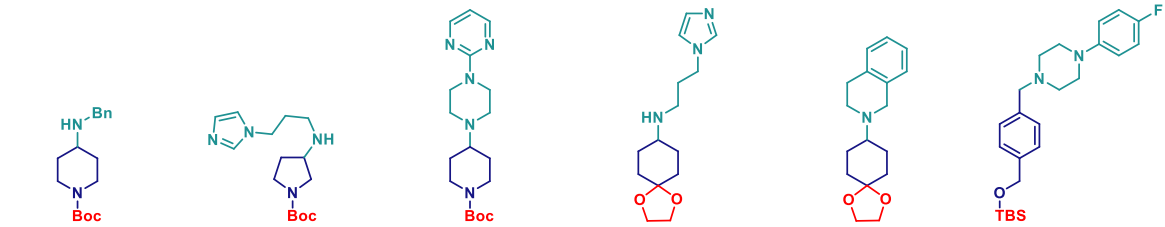
Identified Chemistry Limitation

Known limitations of reductive amination are present in Synple automated reductive amination as well. For example, reactions are sluggish with conjugated ketones such as acetophenone and benzophenone, and other α,β-unsaturated ketones. Sterically hindered ketones also react more slowly and may result in low conversions.

Reactants
At present, the reaction has not been fully optimized for aliphatic aldehydes, weakly nucleophilic or highly hindered amines. The reaction works to a certain level, but conversion and/or purity may be lower. Optimization of these cases is currently ongoing.

Insoluble starting materials
The carbonyl compound (aldehyde or ketone) and amine must be soluble in the reaction solvent (CH_2Cl_2/HFIP 4:1) when the sample is prepared initially. Insoluble materials will lead to low or no conversion, and in the worst case, may cause damage to the synthesizer. During sample preparation, 1.0 mL of MeOH can be added in the vial to improve solubility. MeOH/HFIP (4:1) can be also used in place of CH_2Cl_2/HFIP (4:1) as starting solvent, even though a drop of yield and purity may be observed in that case.

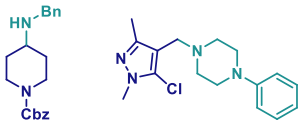
Acid sensitive functional groups
Acid sensitive functional groups, such as *tert*-butoxycarbonyl (Boc) groups or acetals, may undergo partial cleavage during the purification step due to the acidity of SCX. The amount of cleavage might vary from 0 to ~30% for Boc group, and 0 to ~20% for acetals, depending on substrates. For acetals, transacetalization to dimethyl acetal may occur. Silyl groups are less stable, and cleavage will be observed in higher amount. In case of esters, transesterification into methyl ester may be observed in tiny amount. These problems can be avoided by disabling the purification step at the beginning of the sequence (see Reaction Parameter Editing).



57% (+29% Boc cleavage) | 70% (+9% Boc cleavage) | 83% (traces of Boc cleavage) | 82% (+3% dimethyl acetal) | 78% (+20% dimethyl acetal) | 19% (+57% alcohol)

Amine salts
Amine salts do not work directly with the Synple automated reductive amination. Currently, only free amines can be used as the starting materials. A new reductive amination method for amine salts is under development.

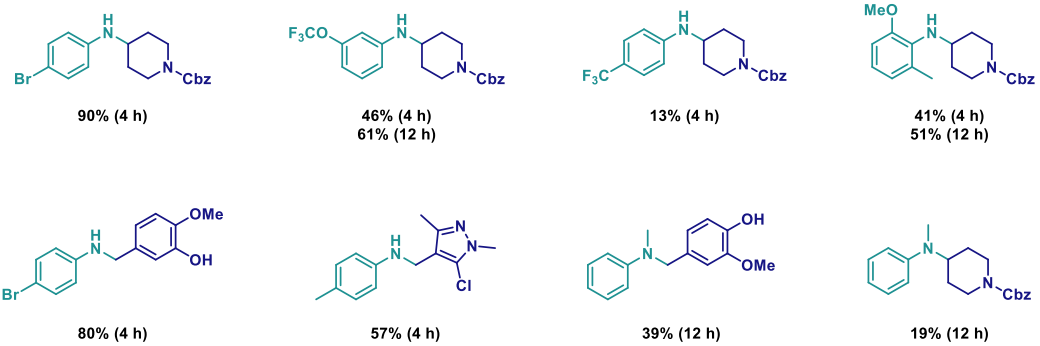
As an alternative, 1.0 equiv of Et_3N can be added to the mixture before starting the reaction. However, this will lead to a drop in yield compared to the reaction using a free amine (yields in parentheses are from reactions using free amines).



58% (89%) | 68% (82%)

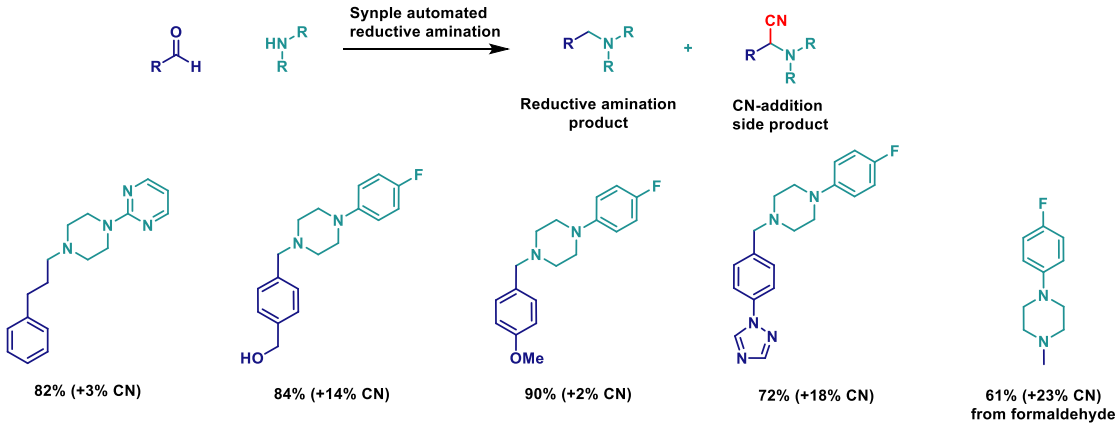
Aryl amines
Synple automated reductive amination is optimized for alkyl amines. Despite this, aryl amines can also be suitable substrates for this application. For the best possible outcome, we suggest to use the *Ketone + secondary amine* sequence when aryl amines are used. Electron-rich and electron-neutral aryl amines react smoothly with both aldehydes and ketones in such conditions, while electron-poor aryl amines will result in very low or no conversion. In such cases conversion may be improved by increasing the reaction time to 12 h (see Reaction Parameter Editing). In most cases, amines do not reach full conversion, therefore addtional

purification is required to obtain pure product. Reactions of secondary aryl amines are generally sluggish both with aldehyde and ketones. Selected examples of products obtained from aryl amines, see below:



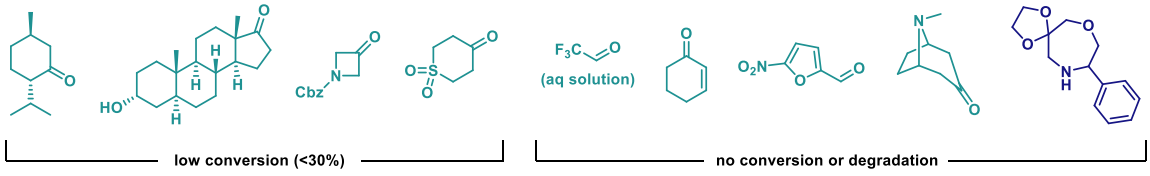
Possible impurities

1) Excess/unreacted starting materials
   The purification method relies on trapping all the basic substances present in the crude mixture, then wash off everything non-basic. Thus, if the conversion of amine is incomplete, it will be recovered at the end together with the product (with the exception of *aldehyde + primary amine*, which includes a primary amine scavenger). For the same reason, if the chosen carbonyl compound (aldehyde or ketone) contains basic moieties, any excess of it will be recovered with the product. In both cases, an additional purification is required to obtain the pure product.
2) CN-addition
   Occasionally the product can contain a small amount (up to ~20%) of impurity, which arises from CN-addition to the imine intermediate. This is a known side reaction when using cyanoborohydride as the reducing agent. This side product is observed more often in the reaction between an aldehyde and a secondary amine and is not able to be removed by our integrated purification system. An additional purification is required to obtain the puer product.. Examples when CN-addtiion side product were observed, see below:



Unsuccessful substrates

Here is a list of failed substrates (low or no conversion) using Synple automated reductive amination, due to either steric or electronic effect.



Reaction Parameter Editing

Editing parameters:

Aldehyde + primary amine / Aldehyde + secondary amine / Ketone and primary amine

Parameter 1	Reaction time for reduction (in seconds)
Parameter 2	Amount of solvent for elution from “catch & release” resin: In cases of highly polar substrates, more solvent could be required to ensure a full recovery of the product from SCX. Therefore, the value can be increased. 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)

Ketone + secondary amine

Parameter 1	Reduction step temperature for cartridge
Parameter 2	Reduction step temperature for reaction vial
Parameter 3	Reaction time for reduction (in seconds)
Parameter 4	Amount of solvent for elution from SCX: 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:

Aldehyde + primary amine

Part 1: Excess amine scavenging
The reaction solution is passed through compartment 4 (polymer-supported benzaldehyde) to scavenge any remaining primary amine. If undesired, the step can be disabled.

Part 2: Purification step
The purification step of the reaction can be disabled. In case of acid sensitive functional groups, the purification might not be suitable. The synthesizer will then provide the reaction product in solution in the reaction vial after the reduction step.

Ketone + secondary amine / ketones + primary amine / aldehyde + secondary amine

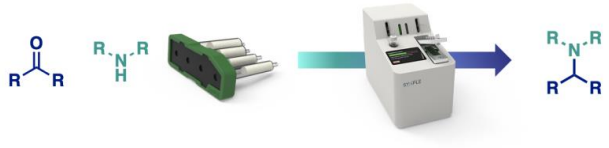
Part 1: Purification step
The purification step of the reaction can be disabled. In case of acid sensitive functional groups, the purification might not be suitable. The synthesizer will then provide the reaction product in solution in the reaction vial after the reduction step.

Reaction Planning

Solubility
Both starting materials shall be soluble in the reaction solvent (see Identified Chemistry Limitation – Insoluble starting materials).

Scale
The Synple automated reductive amination is optimized for a scale of 0.2-0.5 mmol. If less than 0.2 mmol or more than 0.5 mmol of substrate is employed, a low yield of product may be obtained.

Sample Preparation

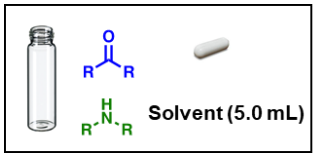


Precautions
The Synple automated reductive amination is not particularly air or moisture sensitive. Still, to ensure a successful reaction is recommended to use the reductive amination cartridge directly after opening. In addition, is suggested to run automated DCM wash before setting up a reductive amination reaction.

Setup

Components for sample preparation:

- Vial
- Aldehyde or ketone
- Amine
- Stirbar
- 5.0 mL solvent (see below)



Guide for solvents and ratios for sample preparation

Aldehyde + primary amine
CH_2Cl_2 (4.0 mL, 99.8%) and HFIP (1.0 mL, 99+%)
For best results, choose a ratio of aldehyde to amine = 1/2

Aldehyde + secondary amine
CH_2Cl_2 (4.0 mL, 99.8%) and HFIP (1.0 mL, 99+%)
For best results, choose a ratio of aldehyde to amine = 1/1 to 2/1

Ketone + primary amine
CH_2Cl_2 (4.0 mL, 99.8%) and HFIP (1.0 mL, 99+%)
For best results, choose a ratio of ketone to amine = 1/1 to 2/1

Ketone + secondary amine
Toluene (5.0 mL, >=99.8%)
Toluene can be replaced by MeOH to improve solubility
For best results, choose a ratio of ketone to amine = 2/1 (1:1 in case of aldehydes and aryl amines)

Machine Solvents for use with Reductive Amination cartridges

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

	S1: CH_2Cl_2, 99.8%, anhydrous, 50 ppm amylene tolerated
	S2: –
	S3: MeOH, HPLC grade
	S4: *N*,*N*-Diispropylamine (175 mL, >=99.5%) in MeOH (325 mL)
	S5: –

For compatibility with the solvent mixutres of other Synple reaction classes MeOH can be replaced here with EtOH or *i*-PrOH

Machine Cleaning after Reductive Amination Reaction

1) Run automated CH_2Cl_2 wash right after the reductive amination reaction.
2) If any solid particles are observed in the lines after the reductive amination reaction, run automated MeOH wash, then an automated CH_2Cl_2 wash.

Solvent Consumption and Run Time

SEQUENCE RUNTIME	
Reaction Sequence	**Time**
A Aldehyde + primary amine	4 h 43 min
B Aldehyde + secondary amine	4 h 21 min
C Ketone + primary amine	4h 21 min
D Ketone + secondary amine	5 h 29 min

SOLVENT COMSUMPTION FOR SEQUENCES A, B, C	
For Reaction Setup	**Amount**
Dichloromethane (CH_2Cl_2)	3 mL
Hexafluoroisopropanol (HFIP)	1 mL
Machine Solvents	
Dichloromethane (CH_2Cl_2)	37 mL
Methanol (MeOH)	40 mL
Diisopropylamine (DIPA) – Methanol (MeOH) mixture (13:7)	19 mL

SOLVENT COMSUMPTION FOR SEQUENCE D	
For Reaction Setup	**Amount**
Toluene	5 mL
Machine Solvents	
Dichloromethane (CH_2Cl_2)	37 mL
Methanol (MeOH)	40 mL
Diisopropylamine (DIPA) – Methanol (MeOH) mixture (13:7)	19 mL