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Supplementary information

Reaction screening in multiwell plates: high-throughput optimization of a Buchwald–Hartwig amination

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Reaction Screening in Multiwell Plates: High-Throughput Optimization of a Buchwald-Hartwig Amination

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Supplementary information

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1. General considerations

1.1 General experimental details

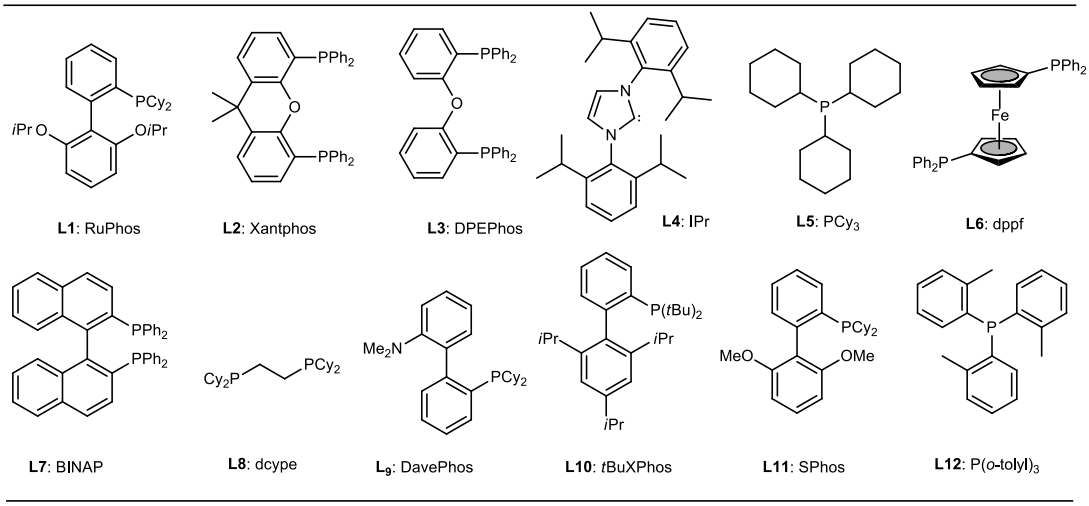
Unless otherwise noted, reactions were conducted under an atmosphere of nitrogen in 1.2 mL 8 x 40 mm glass vials enclosed within an aluminum 96-well plate. 96-well plates were heated on a Heidolph magnetic stirrer/hotplate. Filtration was performed manually by passing 15 µL aliquots of each reaction mixture through a short plug of Silicycle F60 40-63 µm silica gel, making use of a chemically-resistant 96-well filtration plate to filter samples into a second 96-well plate.

1.2 Instrumentation

GC data was obtained via a 5-point calibration curve using FID analysis on an Agilent Technologies 7890B GC with a 30 m x 0.25 mm HP-5 column which was equipped with an XYZ autosampler capable of accommodating multiwell plates. ^{1}H NMR and ^{13}C NMR were recorded on a Bruker AVANCE 400 MHz spectrometer. ^{1}H NMR spectra were internally referenced to the residual solvent signal (e.g., $CDCl_3$ = 7.27 ppm). ^{13}C NMR spectra were internally referenced to the residual solvent signal (e.g., $CDCl_3$ = 77.00 ppm). Data for ^{1}H NMR are reported as follows: chemical shift (δ ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, quin = quintet, m = multiplet), coupling constant (Hz), integration.

1.3 Materials

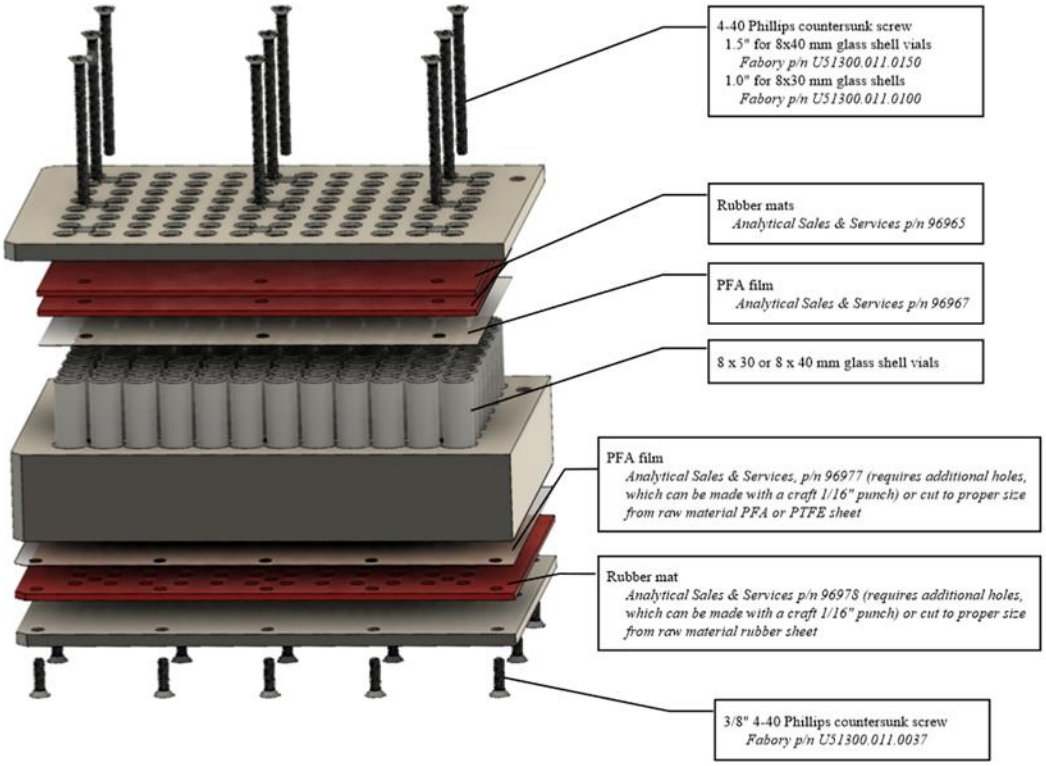
Organic solvents were purified by rigorous degassing with nitrogen before passing through a PureSolv solvent purification system. Low water content was confirmed by Karl Fischer titration (<20 ppm for all solvents). All starting materials, bases and ligands (**Supporting Figure 1**) were obtained commercially from Sigma-Aldrich, Alfa Aesar or Combi-Blocks and used as received. $[Pd(cinnamyl)Cl]_2$ (97% purity) was purchased from Sigma-Aldrich.



Supporting Figure 1. Structures of ligands screened in this protocol

1.4 96-well plate

Each 96-well plate consists of three main parts – the body of the plate, the cover of the plate and the bottom of the plate. The bottom plate is screwed to the main body of the plate, and the cover of the plate is screwed in to secure the reaction vials. Between each of these three main parts lies a rubber gasket and a PFA sheet in order to provide support to the reaction vials and to ensure a proper seal between the vials and the aluminum plate (**Supporting Figure 2**).



Supporting Figure 2. Dissecting the anatomy of a 96-well plate

A 3D file for the construction of a 96-well plate out of a 4” by 6” aluminum block is provided in the file titled *Supplementary Data 02*. 96-well plates may also be purchased from Analytical Sales & Services *(catalogue number 96973*).

2. Experimental procedures

General

Three different 96-well plates were prepared and analyzed according to the protocol steps **1**-**63** – Plate A, B and C. Plates A and B were both run using a Heidolph heater/stir plate and gravity filtration for purification. Plate C was run using a tumble stirrer and pressurized parallel filtration device. All yields (**Supporting Figures 3-5**) were obtained via a 5-point calibration curve using GC analysis with 1,3,5-trimethoxybenzene as an internal standard.

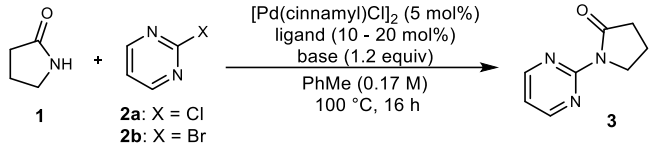
Scale-up

In order to confirm that results obtained using high-throughput experimentation could be replicated on the bench, the highest yielding reaction was repeated in round-bottom flasks at the 1.0 mmol scale.

To an oven-dried 50 mL round-bottom flask was added one 100 mm PTFE-coated stir bar. The round-bottom flask was then charged with 0.05 mmol of $[Pd(cinnamyl)Cl]_2$ (26 mg), 0.1 mmol of Xantphos (58 mg), 1.2 mmol of Cs_2CO_3 (391 mg) and 1.2 mmol of 2-bromopyrimidine (191 mg). 6 mL of anhydrous PhMe was added to the round-bottom flask and the resulting solution was stirred at 650 RPM. 1.0 mmol of 2-pyrrolidone (76 µL) was added before equipping the round-bottom flask with a reflux condenser and argon-filled balloon. The reaction mixture was then heated to 100 °C for 16 hours. Upon completion, the reaction mixture was allowed to come to room temperature before being quenched with 10 mL distilled H_2O. 25 mL of ethyl acetate was added to the reaction mixture before it was transferred to a separatory funnel. This solution was washed 2 times with 10 mL of $NaHCO_3$ and once with sat. NaCl (aq). The organic fractions were dried with $MgSO_4$ and the resulting mixture was filtered via suction filtration into a 250 mL round-bottom flask – solvent was subsequently removed in vacuo using a rotary evaporator to afford crude product. Column chromatography performed using a CombiFlash Rf+ instrument eluted product using a gradient of 25-60% ethyl acetate in hexanes.

3. Experimental results

3.1 Yields associated with this protocol



	L1	L2	L3	L4	L5	L6	L7	L8	L9	L10	L11	L12	
B1	21	71	59	18	11	65	68	64	17	31	18	4	**2a**
B2	23	83	67	14	17	69	73	71	13	33	27	6	
B3	6	29	21	0	4	18	37	20	0	4	8	0	
B4	0	2	5	0	0	7	3	4	0	0	0	0	
B1	17	73	65	21	11	60	70	67	14	27	21	3	**2b**
B2	22	81	73	17	9	74	67	71	21	18	14	13	
B3	4	34	19	0	0	14	19	3	0	13	9	0	
B4	0	8	3	0	0	4	6	0	0	0	0	0	

Supporting Figure 3. Yields acquired for Plate A using a hotplate to heat and stir reactions, as described in the protocol steps **1-63**.

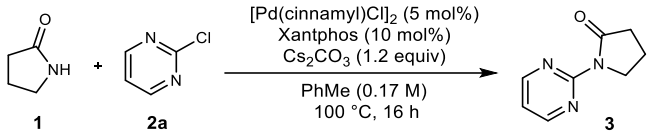
	L1	L2	L3	L4	L5	L6	L7	L8	L9	L10	L11	L12	
B1	23	73	53	17	14	69	66	62	8	34	21	2	**2a**
B2	27	85	72	5	17	72	71	74	23	37	29	4	
B3	3	37	24	0	9	24	31	23	3	2	7	0	
B4	0	4	4	0	0	8	2	7	0	0	0	0	
B1	12	78	57	16	18	57	69	66	13	24	27	6	**2b**
B2	18	83	81	24	4	72	55	79	28	19	24	19	
B3	8	31	8	0	0	14	23	10	0	13	13	0	
B4	0	13	2	0	0	3	7	0	0	0	0	0	

Supporting Figure 4. Yields acquired for Plate B, a replication of Plate A.

	L1	L2	L3	L4	L5	L6	L7	L8	L9	L10	L11	L12	
B1	27	74	55	14	13	63	62	71	15	32	15	3	**2a**
B2	28	83	71	11	26	74	77	68	12	36	31	8	
B3	11	26	18	0	2	17	41	7	0	4	6	0	
B4	0	5	3	0	0	4	3	3	0	0	0	0	
B1	19	71	62	17	7	56	69	68	19	23	24	6	**2b**
B2	20	84	71	12	14	75	64	71	18	19	16	14	
B3	5	32	17	0	0	14	24	8	0	9	7	0	
B4	0	6	2	0	0	7	4	0	0	0	0	0	

Supporting Figure 5. Yields acquired for Plate C, using a tumble stirrer to heat and stir reactions

Scale-up experiment



1-(2-Pyrimidinyl)-2-pyrrolidinone (**3**) was prepared according to the aforementioned scale-up procedure. Column chromatography performed on a Combiflash Rf+ automated chromatography instrument using a gradient of 25-60% ethyl acetate in hexanes. Product was recovered as a white solid (87 mg, 71% yield). NMR data matched those reported previously.[1] **^{1}H NMR** ($CDCl_3$): 8.65 (d, *J* = 5.1 Hz, 2H), 7.05 (t, *J* = 5.1 Hz, 1H), 4.33-4.07 (m, 2H), 2.71 (t, *J* = 7.9 Hz, 2H), 2.16 (m, 2H). **Accurate mass (EI)**: Theoretical: 163.0714. Found: 163.0718. Spectral Accuracy: 97.3%. **Melting Point:** 109-112 °C.

3.2. Analysis of results

3.2. i. Reproducibility of using a hot plate to heat/stir reactions vs. using a tumble stirrer

There are several sources of experimental error in the high-throughput protocol. For example, the use of polypropylene transfer scoops to transfer solids and the different heating/stirring setups between plates A/B (traditional stir plate) and plate C (heated tumble stirrer designed for multiwell plates) can lead to variance in yields. The analysis of reproducibility data is provided below (**Supporting Table 1, Supporting Figures 6-8**). Good agreement is observed between the triplicate data points in most cases. Notably, data obtained using protocol steps **1**-**63** (plates A and B) was indistinguishable from data obtained with the costlier equipment used for running place C.

Supporting Figure 6 provides an analysis of the range in yields that were obtained from this protocol. As an example, the experiment conducted in well A3 provided a yield of 59% in plate A, 53% in plate B and 55% in plate C, providing a range of 6%. The vast majority of yields were within 5% of one another among all of the three plates, with none of the 96 experimental triplicates having a range in yields of greater than 20%.

The standard deviations for each of the triplicate experiments were calculated and found to range from 0 to 8.5 (**Supporting Table 1**). The average (pooled) standard deviation was found to be ± 3.0%, providing an appropriate metric for the error bar of any individual experiment.

range of yields	number of experiments
0	22
1-5%	37
6-10 %	30
11-19%	7
>20%	0
avg. range	4.4
avg. standard deviation	± 3.0 %

Supporting Figure 6. Tabulating the range of yields amongst plates A, B and C.

experiment	plate A, yield (%)	plate B, yield (%)	plate C, yield (%)	st. dev.	(st. dev.)^2	avg. st. dev	Σ(st.dev)^2	Σ(st.dev)^2/ N	pooled standard deviation
A1	21	23	27	3.1	9.3	3.01	872.7	9.090	3.015
A2	23	27	28	2.6	7.0				
A3	6	3	11	4.0	16.3				
A4	0	0	0						
A5	17	12	19	3.6	13.0				
A6	22	18	20	2.0	4.0				
A7	4	8	5	2.1	4.3				
A8	0	0	0						
A9	71	73	74	1.5	2.3				
A10	83	85	83	1.2	1.3				
A11	29	37	26	5.7	32.3				
A12	2	4	5	1.5	2.3				
B1	73	78	71	3.6	13.0				
B2	81	83	84	1.5	2.3				
B3	34	31	32	1.5	2.3				
B4	8	13	6	3.6	13.0				
B5	59	53	55	3.1	9.3				
B6	67	72	71	2.6	7.0				
B7	21	24	18	3.0	9.0				
B8	5	4	3	1.0	1.0				
B9	65	57	62	4.0	16.3				
B10	73	81	71	5.3	28.0				
B11	19	8	17	5.9	34.3				
B12	3	2	2	0.6	0.3				
C1	18	17	14	2.1	4.3				
C2	14	5	11	4.6	21.0				
C3	0	0	0						
C4	0	0	0						
C5	21	16	17	2.6	7.0				
C6	17	24	12	6.0	36.3				
C7	0	0	0						
C8	0	0	0						
C9	11	14	13	1.5	2.3				
C10	17	17	26	5.2	27.0				
C11	4	9	2	3.6	13.0				
C12	0	0	0						
D1	11	18	7	5.6	31.0				
D2	9	4	14	5.0	25.0				
D3	0	0	0						
D4	0	0	0						
D5	65	69	63	3.1	9.3				
D6	69	72	74	2.5	6.3				
D7	18	24	17	3.8	14.3				
D8	7	8	4	2.1	4.3				
D9	60	57	56	2.1	4.3				
D10	74	72	75	1.5	2.3				
D11	14	14	14						
D12	4	3	7	2.1	4.3				

$$Pooled\ Standard\ Deviation = \sqrt{\frac{(S_A^2 + S_B^2 \ldots + S_N^2)}{N}}$$

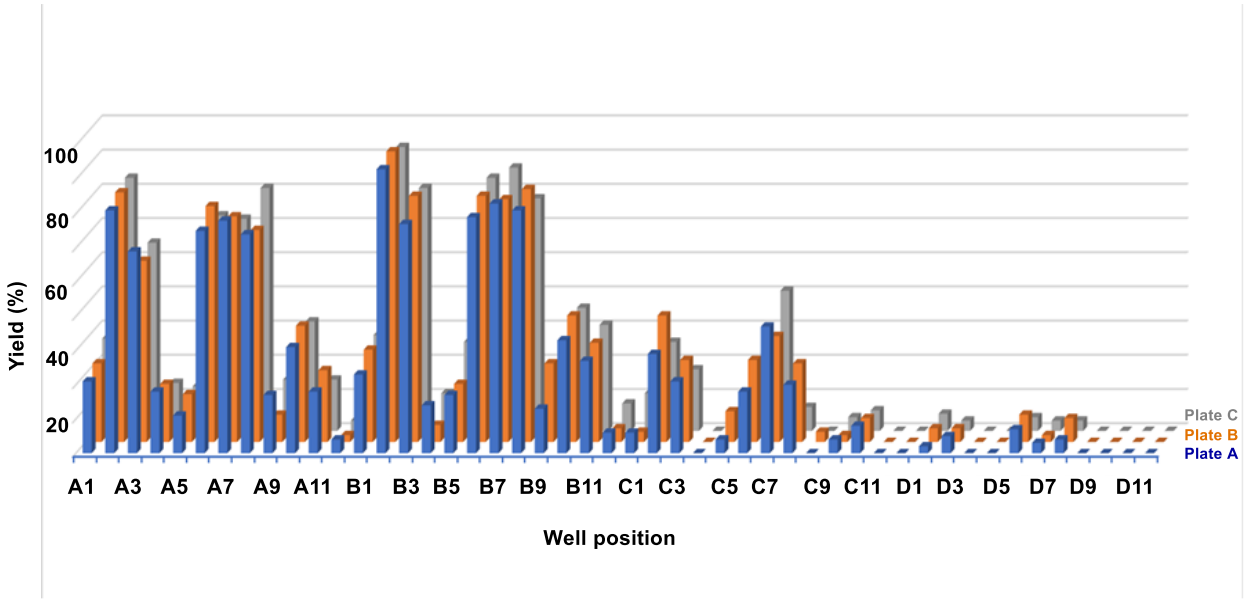
$$N = 96$$

$$S = Standard\ deviation$$

experiment	plate A, yield (%)	plate B, yield (%)	plate C, yield (%)	st. dev.	(st. dev.)^2	avg. st. dev	Σ(st.dev)^2	Σ(st.dev)^2 / N	pooled standard deviation
E1	68	66	62	3.1	9.3				
E2	73	71	77	3.1	9.3				
E3	37	31	41	5.0	25.3				
E4	3	2	3	0.6	0.3				
E5	70	69	69	0.6	0.3				
E6	67	55	64	6.2	39.0				
E7	19	23	24	2.6	7.0				
E8	6	7	4	1.5	2.3				
E9	64	62	71	4.7	22.3				
E10	71	74	68	3.0	9.0				
E11	20	23	7	8.5	72.3				
E12	4	7	3	2.1	4.3				
F1	67	66	68	1.0	1.0				
F2	71	79	71	4.6	21.3				
F3	3	10	8	3.6	13.0				
F4	0	0	0						
F5	17	8	15	4.7	22.3				
F6	13	23	12	6.1	37.0				
F7	0	3	0	1.7	3.0				
F8	0	0	0						
F9	14	13	19	3.2	10.3				
F10	21	28	18	5.1	26.3				
F11	0	0	0						
F12	0	0	0						
G1	31	34	32	1.5	2.3				
G2	33	37	36	2.1	4.3				
G3	4	2	4	1.2	1.3				
G4	0	0	0						
G5	27	24	23	2.1	4.3				
G6	18	19	19	0.6	0.3				
G7	13	13	9	2.3	5.3				
G8	0	0	0						
G9	18	21	15	3.0	9.0				
G10	27	29	31	2.0	4.0				
G11	8	7	6	1.0	1.0				
G12	0	0	0						
H1	21	27	24	3.0	9.0				
H2	14	24	16	5.3	28.0				
H3	9	13	7	3.1	9.3				
H4	0	0	0						
H5	4	2	3	1.0	1.0				
H6	6	4	8	2.0	4.0				
H7	0	0	0						
H8	0	0	0						
H9	3	6	6	1.7	3.0				
H10	13	19	14	3.2	10.3				
H11	0	0	0						
H12	0	0	0						

Supporting Table 1: Investigating the standard deviation of the yields associated with this high-throughput protocol

Supporting Figure 7 provides a graphical representation of the yields across the three plates for the experiments conducted with 2-chloropyrimidine. Supporting Figure 8 provides an analysis of the yield distribution associated with the Buchwald-Hartwig amidation reaction between 2-pyrrolidone and a 2-halopyrimidine.



Supporting Figure 7. Comparing the % yield amongst Plates A, B and C for the experiments performed with 2-chloropyrimidine

yield distribution			
% yield	**plate A**	**plate B**	**plate C**
90+	0	0	0
80-89	2	3	2
66-79	12	12	12
51-65	4	5	6
25-49	7	9	9
16-24	18	17	15
1- 15	31	29	30
0	22	21	22

Supporting Figure 8. Tabulating the % yield distribution amongst plates A, B and C

3.2. ii. Interpretation of results

Some general observations can be made from the obtained data. We found that bidentate ligands afforded higher yields than monodentate ligands (**Supporting Figure 9**).

monodentate ligands			
	plate A	plate B	plate C
average yield (%)	9	10	10
bidentate ligands			
	plate A	plate B	plate C
average yield (%)	41	42	41

Supporting Figure 9. Comparing the yields acquired while employing mono vs. bi-dentate ligands

We found Xantphos to be the best performing ligand with an average yield of 49%, while IPr, PCy_3, DavePhos and P(*o*-tolyl)$_3$ were the worst, affording 10% yield on average (**Supporting Figure 10**).

ligand	**average yield (%)**			
	plate A	plate B	plate C	overall average
RuPhos	12	11	14	12
Xantphos	48	51	47	49
DPEPhos	39	38	37	38
IPr	9	8	7	8
PCy_3	7	8	8	8
dppf	39	40	39	39
BINAP	43	41	43	42
dcype	38	40	37	38
DavePhos	8	9	8	8
***t*BuXPhos**	16	16	15	16
SPhos	12	15	12	13
P(*o*-tolyl)$_3$	3	4	4	4

Supporting Figure 10. Comparing the yields acquired while employing each of the twelve tested ligands

Both NaO^tBu and Cs_2CO_3 were found to be effective in this reaction (**Supporting Figure 11**). While the use of KHMDS allowed reactivity to proceed to some extent, the use of DBU appeared to shut down reactivity in almost all cases.

base	average yield (%)			
	plate A	plate B	plate C	overall average
NaO^tBu	37	37	37	37
Cs_2CO_3	41	43	42	42
KHMDS	11	12	10	11
DBU	2	2	2	2

Supporting Figure 11. Comparing the yields acquired while employing each of the four tested bases

Lastly, we sought to compare bromo- and chloropyrimidine (**Supporting Figure 12**). While 2-bromopyrimidine afforded higher average yields, the difference between these two electrophiles was minimal.

2-bromopyrimidine			
	plate A	plate B	plate C
average yield	23%	24%	24%
2-chloropyrimidine			
	plate A	plate B	plate C
average yield	20%	21%	22%

Supporting Figure 12. Comparing the yields acquired while employing each of the tested halopyrimidines

The highest yielding reaction featured Cs_2CO_3 as a base, Xantphos as a ligand and 2-bromopyrimidine as an electrophile.

3.2. iii. Reproducibility of polypropylene transfer scoops

Seeking to develop a low-cost approach to high-throughput experimentation in chemistry, we recognized the necessity of expensive solid-handling robots as the primary prohibiting factor. We, however, imagined that inexpensive polypropylene transfer scoops could be utilized in place of these robots, provided that they allowed for reproducible dosing of solid reagents of different densities and constitution.

Seeking to investigate the reproducibility associated with using polypropylene transfer scoops to dose the reagents used in this protocol, we took the mass of 10 different doses of each of the four solids dosed as such in the protocol (**Supporting Figures 13-16**). Each scoop was plunged into a vial containing the desired solid – while removing it, the scoop was brushed against the side of the vial to ensure that the solid was flush with the rim of the scoop. Using this technique, we found that the scoops were quite reproducible, with standard deviations falling in the range of ± 0.31 – 0.85 mg.

dose	weight (mg)	average weight (mg)	Standard deviation	%RSD
1	2.403	2.549	0.3174	12.5 %
2	2.813			
3	2.157			
4	2.439			
5	2.946			
6	2.371			
7	3.105			
8	2.681			
9	2.283			
10	2.297			

Supporting Figure 13: Investigating reproducibility while weighing $[Pd(cinnamyl]Cl]_2$ using a small yellow polypropylene transfer scoop

dose	weight (mg)	average weight (mg)	Standard deviation	%RSD
1	12.846	13.084	0.786	6.0 %
2	12.589			
3	11.934			
4	13.58			
5	14.341			
6	13.284			
7	12.947			
8	13.594			
9	11.944			
10	13.785			

Supporting Figure 14: Investigating reproducibility while weighing NaO^tBu using an extra-large red polypropylene transfer scoop

dose	weight (mg)	average weight (mg)	Standard deviation	%RSD
1	13.472	12.922	0.8475	6.6 %
2	11.894			
3	12.385			
4	12.485			
5	14.194			
6	13.294			
7	11.948			
8	12.375			
9	12.98			
10	14.194			

Supporting Figure 15: Investigating reproducibility while weighing Cs_2CO_3 using an extra-large red polypropylene transfer scoop

dose	weight (mg)	average weight (mg)	Standard deviation	%RSD
1	9.456	8.903	0.7428	8.3 %
2	8.485			
3	8.682			
4	8.957			
5	10.045			
6	7.982			
7	8.958			
8	7.596			
9	9.483			
10	9.386			

Supporting Figure 16: Investigating reproducibility while weighing KHMDS using a large blue polypropylene transfer scoop.

3.2. iv. Reproducibility of micropipettes

Seeking to investigate the reproducibility associated with using a 10-100 µL single-channel micropipette to dose reagents, we took the mass of each of 10 different 100 µL doses of toluene (**Supporting Figure 17**). It is important to note that the volume dispensed by pipettes depends strongly on the properties of the liquid it is dispensing, particularly the liquid's vapour pressure – using other organic solvents will necessitate calibration in order to ensure delivery of accurate volumes. We performed the same tests using a 30-300 µL multichannel pipette – for these data points the average mass of each of the 12 deliveries was obtained (**Supporting Figure 18**). We found that both micropipettes were accurate and precise, with the single-channel micropipette proving to be slightly more precise than the multichannel micropipette, and the multichannel pipette proved to be slightly more accurate.

dose	true volume (mL)	average volume (mL)	standard deviation	%RSD
1	100.31	100.32	0.274	0.27 %
2	100.32			
3	100.18			
4	100.43			
5	100.27			
6	99.94			
7	100.12			
8	100.19			
9	100.53			
10	100.95			

Supporting Figure 17: An analysis of the reproducibility of micropipettes when dosing 100 µL of toluene with a single-channel micropipette

dose	true volume (mL)	average volume (mL)	standard deviation	%RSD
1	100.14	99.96	0.791	0.79 %
2	100.24			
3	99.85			
4	98.99			
5	99.43			
6	100.15			
7	100.32			
8	98.59			
9	100.4			
10	100.53			

Supporting Figure 18: An analysis of the reproducibility of micropipettes when dosing 100 µL of toluene with a multi-channel pipette

4. References

1) Vimolratana, M.; Simard, J.L.; Brown, S.P. Palladium-catalyzed amidation of 2-chloropyrimidines. *Tetrahedron Letters* **2011**, 52, 1020-1022.

5. 96-well plate blueprints

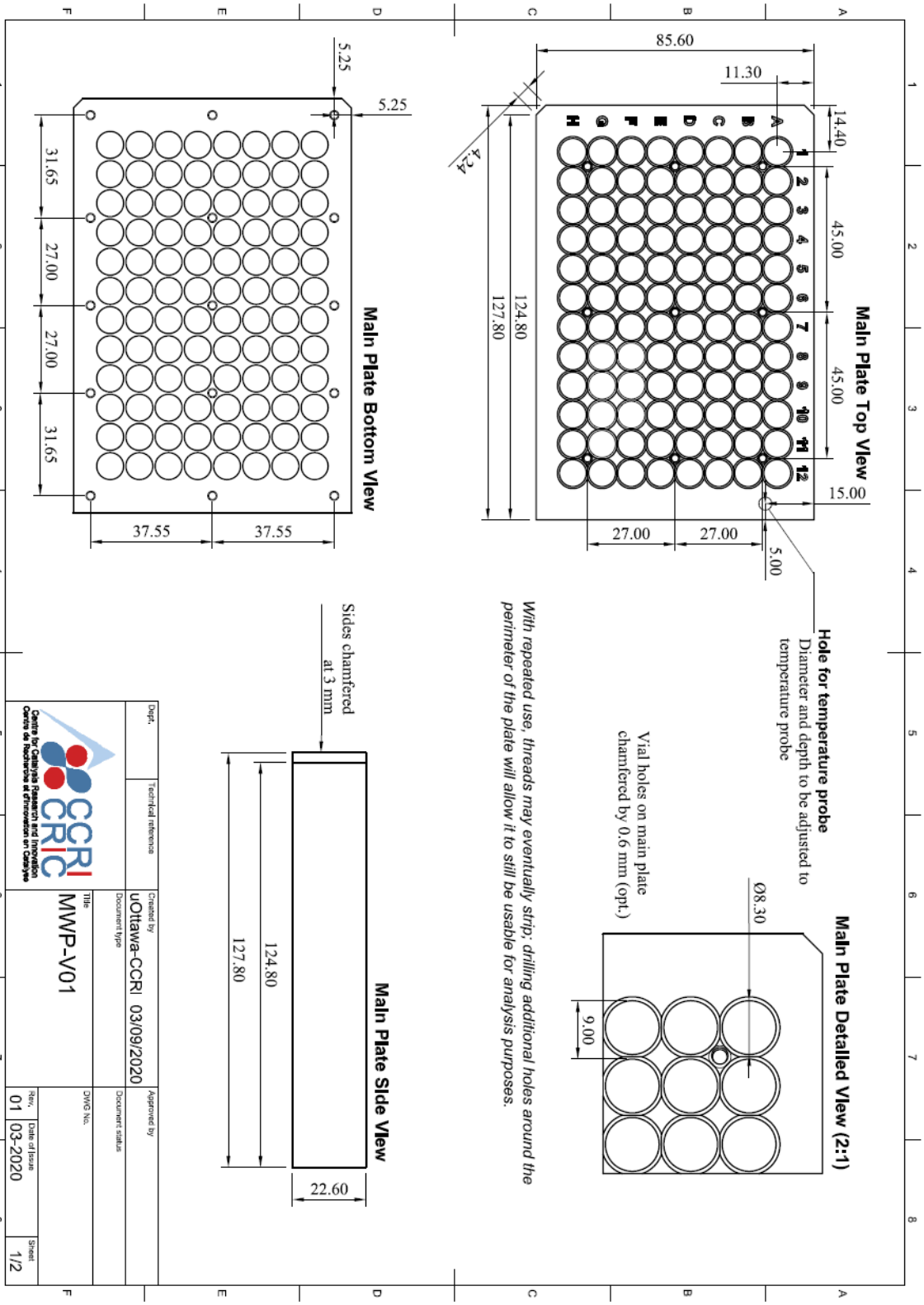
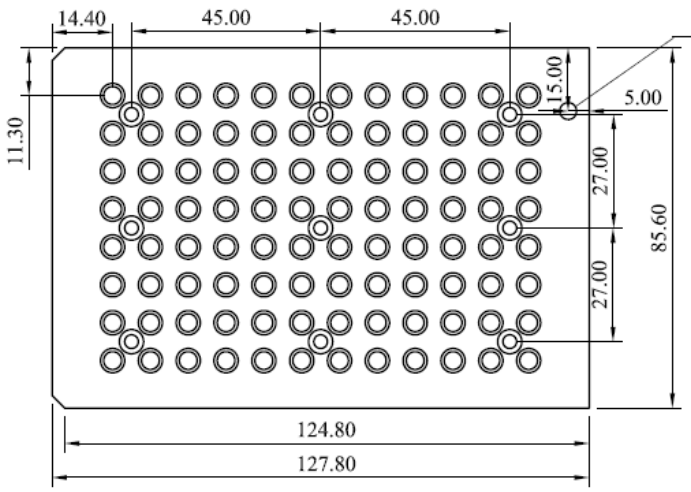
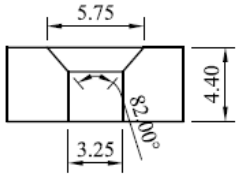


Plate Cover



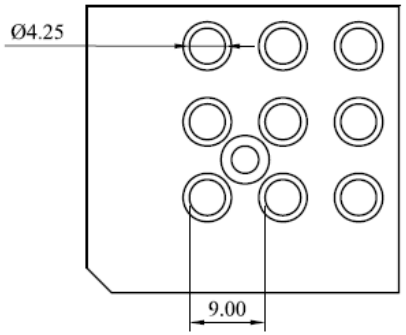
Hole for temperature probe
Diameter and depth to be adjusted to temperature probe

Screw Hole Details Plate Cover (4:1)



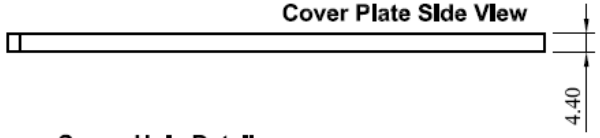
Fabory U51300.011.0100
Fabory U51300.011.0150

Cover Plate Detailed View (2:1)

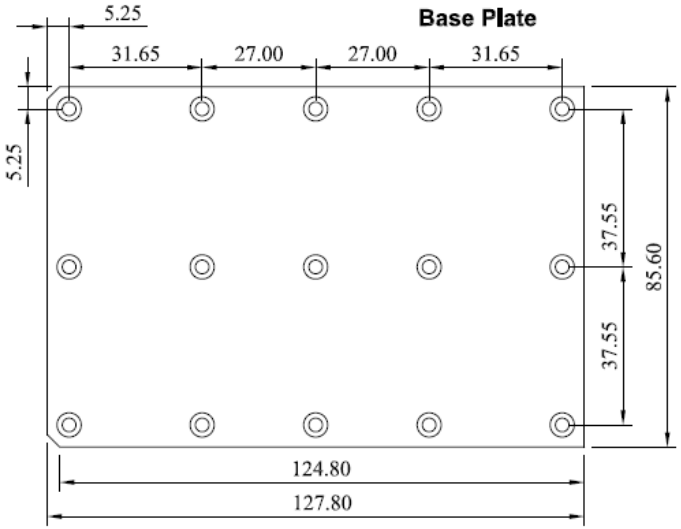


Holes on cover chamfered by 0.75 mm (opt.)

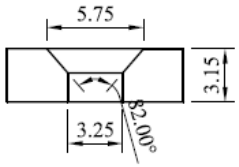
Cover Plate Side View



Base Plate

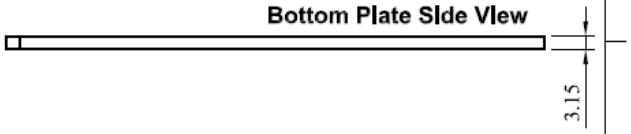


Screw Hole Details Bottom Plate (4:1)



Fabory 51300.011.0037

Bottom Plate Side View



Ensure screws end up flush with the base plate

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