SUPPLEMENTARY INFORMATION

Development of an Automated Kinetic Profiling System with Online HPLC for Reaction Optimization

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Contents

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1. General Remarks

1.1. Chemical Suppliers

Methyl 3-cyclopropyl-3-oxopropanoate **1** (Accela SY001027) and (3-(benzyloxy)phenyl)boronic acid **4** (Acros 358860250) were purchased from commercial sources and used without further purification. Pd-dppf-G3 **5** (Cat# 804983) and Pd-PPh_3-G2 **11** (Cat# 752762) were purchased from Millipore Sigma; Pd(dppf)Cl_2·CH_2Cl_2 (Cat# 46-0450) was purchased from Strem Chemicals. All reactions requiring anhydrous or air-free conditions were run in anhydrous solvents purchased from Millipore Sigma.

1.2. Equipment

Automated reaction monitoring was carried out on a Chemspeed Swing liquid handling robot equipped with an adjustable-pitch four needle (0.8 mm ID) liquid dispense tool, a V&P Scientific tumble stirrer and a Huber Unistat 82T chiller. The robot was integrated with an Agilent 1100 HPLC-UV system through installation of a two position, 6-port HPLC valve on the Chemspeed Swing deck and connection to the Agilent 1100 through 0.17 mm ID tubing. Bilateral communication was established between the Chemspeed Autosuite software and the HPLC valve. Also, unilateral communication was established between the Chemspeed Autosuite software and the Agilent 1100 HPLC-UV (Figure S1).

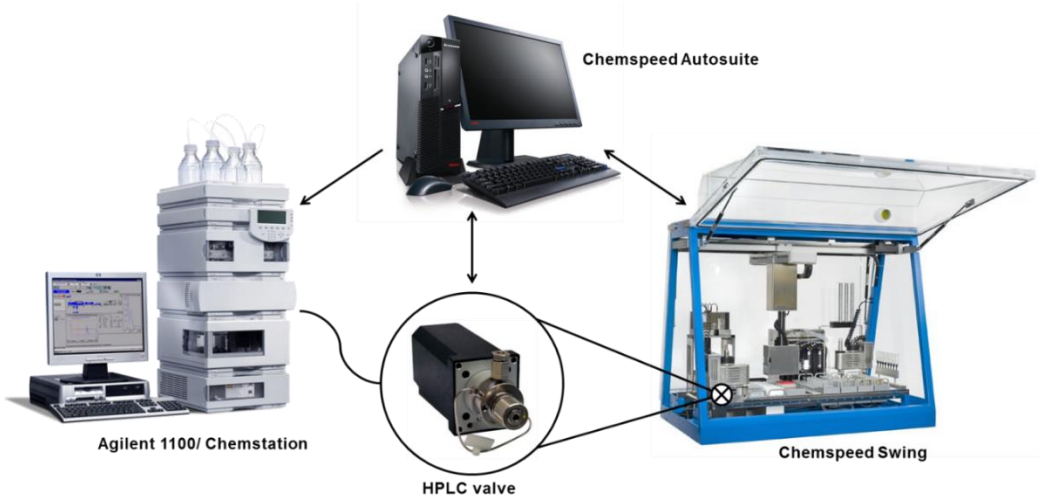


Figure S1: Schematic of the Chemspeed Swing Agilent 1100 integration

Reactions were carried out in sealed 4 ml borosilicate glass vials with red pressure relief caps (CG-4912-01). Vigorous agitation was achieved with parylene-coated NdFeB magnetic tumble stir discs (VP 782N6). Reaction aliquots were sampled into 96-well dilution plates (Analytical Sales 17P687) capped with pre-slit silicone/PTFE cap mats (Analytical Sales 965075).

1.3. Analytical Methods

Column: Imtakt Unison UK-C18 3 µm 10x2 mm (UK020)
Column Temperature: 55 °C
pH 3.5 Ammonium Formate Buffer: 12.6 g ammonium formate + 7.9 ml formic acid to 1 L water
Mobile Phase A: 40 ml pH 3.5 ammonium formate buffer + 3960 ml Water
Mobile Phase B: 40 ml pH 3.5 ammonium formate buffer + 360 ml Water + 3600 ml ACN
Injection volume: 20 µl
Wavelength Monitored: 210 nm

Time (min)	Flow (ml/min)	%MPA	%MPB
0.00	1.00	95.0	5.0
3.50	1.00	0.0	100.0
3.90	1.00	0.0	100.0
3.91	1.00	95.0	5.0
4.00	1.00	95.0	5.0

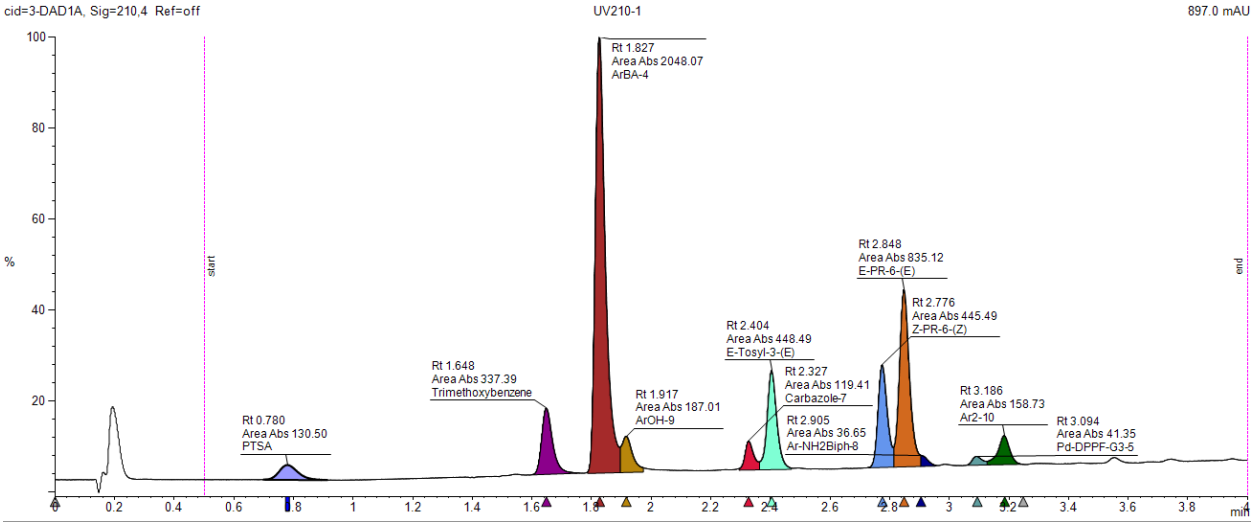


Figure S2: Representative HPLC trace

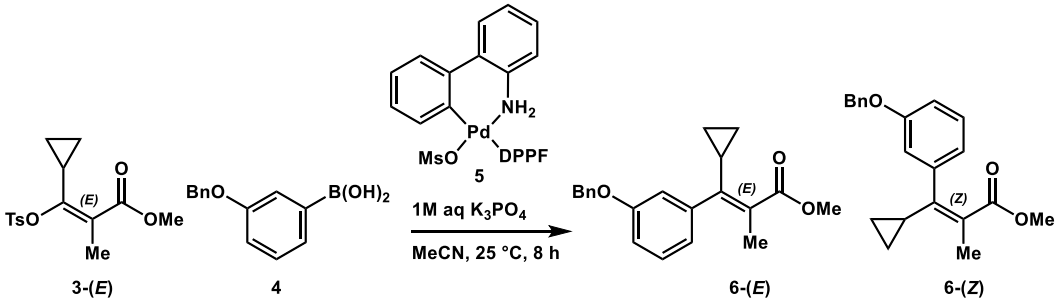
Retention times of quantified reaction components:

Name	Retention Time
1,3,5-Trimethoxybenzene **IS**	1.65 min
Aryl boronic acid **4**	1.83 min
Phenol **9**	1.92 min
Carbazole **7**	2.33 min
E-Tosylate **3-(*E*)**	2.40 min

Z Suzuki Product **6-(*Z*)**	2.78 min
E Suzuki Product **6-(*E*)**	2.85 min
Aryl Aminobiphenyl Adduct **8**	2.91 min
Pd-dppf-G3 Precatalyst **5**	3.09 min
Biaryl **10**	3.19 min

2. Experimental Procedures

2.1. Procedures for Automated Kinetic Profiling



Reaction set-up procedure 1 (Figures 2, 3 and 4): To a 4 ml reaction vial equipped with a magnetic tumble stir disc under N_2 atmosphere was charged (*E*)-methyl 3-cyclopropyl-2-methyl-3-(tosyloxy)acrylate **3-(*E*)** (40.0 mg, 0.129 mmol), (3-(benzyloxy)phenyl)boronic acid **4** (32.3 mg, 0.142 mmol), palladium precatalyst **5** (5.96 mg, 6.44 µmol) and 1,3,5-trimethoxybenzene (2.17 mg, 0.013 mmol). ACN (1000 µl) and water (10 µl) were added and the mixture agitated to dissolve all solids. To a 40 ml vial was charged K_3PO_4 (2.12 g, 10.0 mmol) and water to dilute to 10 ml. The resulting 1 M aqueous K_3PO_4 solution was then sparged with N_2 gas for 30 min in preparation for automated dispensing (387 µl, 0.387 mmol). Finally, to a 96-well sample dilution plate was charged 400 µl of a 9:1 mixture of acetonitrile and aqueous pH 3.5 ammonium formate buffer.[i]

Note for Figure 4b: $Pd(dppf)Cl_2 \cdot CH_2Cl_2$ (5.26 mg, 6.44 µmol) utilized instead of precatalyst **5**.

Reaction set-up procedure 2 (Figure 5): To a 4 ml reaction vial equipped with a magnetic tumble stir disc under N_2 atmosphere was charged 250 µl of a 400 mM solution of (*E*)-methyl 3-cyclopropyl-2-methyl-3-(tosyloxy)acrylate **3-(*E*)** in ACN (31.0 mg, 0.100 mmol), 250 µl of a 440 mM solution of (3-(benzyloxy)phenyl)boronic acid **4** in ACN (25.1 mg, 0.110 mmol), 250 µl of a 4 mM solution of $Pd(dppf)Cl_2 \cdot CH_2Cl_2$ in ACN (0.817 mg, 1.00 µmol) and 100 µl of a 100 mM solution of 1,3,5-trimethoxybenzene in ACN (1.68 mg, 0.010 mmol). Water (10 µl) was added and the mixture agitated to dissolve all solids. To a 40 ml vial was charged K_3PO_4 (2.12 g, 10.0 mmol) and water to dilute to 10 ml. The resulting 1 M aqueous K_3PO_4 solution was then sparged with N_2 gas for 30 min in preparation for automated dispensing (300 µl, 0.300 mmol). Finally, to a 96-well

sample dilution plate was charged 400 µl of a 9:1 mixture of acetonitrile and aqueous pH 3.5 ammonium formate buffer.[i]

Note for Figure 5b: Dispensed 250 µl of an 8 mM solution of $Pd(dppf)Cl_2 \cdot CH_2Cl_2$ in ACN (1.63 mg, 2.00 µmol) instead of a 4 mM solution.

Automated sampling procedure: The reaction vial, aqueous K_3PO_4 vial and dilution plate were sealed and placed on the Chemspeed Swing robot deck for automated initiation, sampling, dilution and HPLC injection (Figure S3). The robot deck was inerted under positive pressure N_2 atmosphere, tumble stirring was initiated (300-500 rpm), and the reaction vial temperature set to 25 °C. To the reaction vial was transferred 1M aqueous K_3PO_4 by needle. This was immediately followed by transfer of a 20 µl aliquot from the reaction vial to the dilution plate by needle. Upon transfer, a 200 µl N_2 cushion was drawn by the needle, followed by a 200 µl aliquot from the dilution plate. The 200 ul aliquot and N_2 cushion were then re-dispensed to the dilution plate at 10 ml/min flow rate to effectively mix the sample. A 50 µl aliquot was then transferred from the dilution plate to the injection port of the HPLC selection valve, over-filling the 20 µl loop with diluted sample. Once the loop was filled, the valve was switched and the HPLC run triggered, allowing the HPLC pump to push the mobile phase through the loop, moving the sample into the HPLC column. The valve was then rinsed with 1000 µl of acetonitrile. This sampling, dilution and injection procedure was repeated every 5-20 minutes until reaction completion.

Note for Figure 3: 300 rpm agitation rate; HPLC injection repeated after a 20 h hold. Note for Figures 4 and 5: 500 rpm agitation rate.

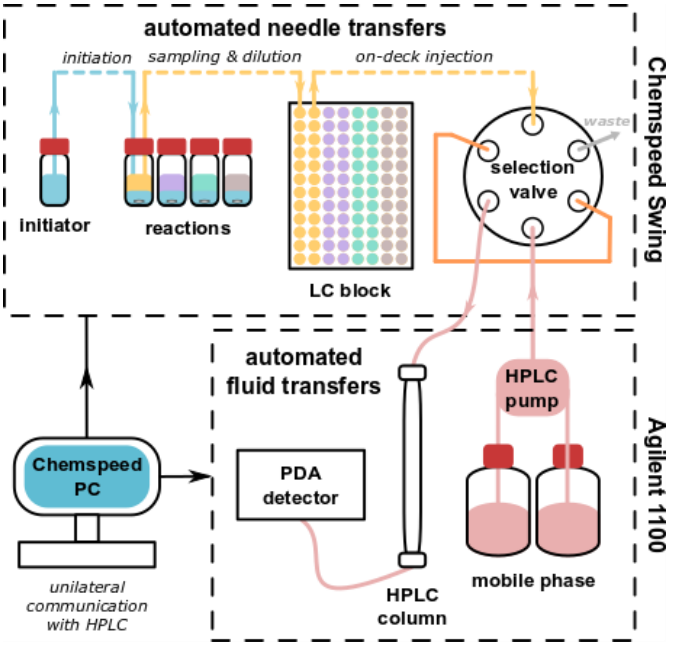
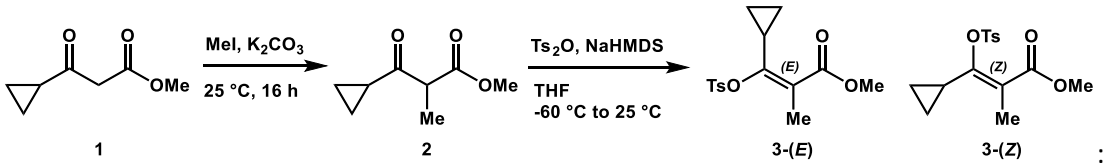


Figure S3: Schematic of the Automated Kinetic Profiling System with Online HPLC

2.2. Procedures for Preparation of Reaction Components

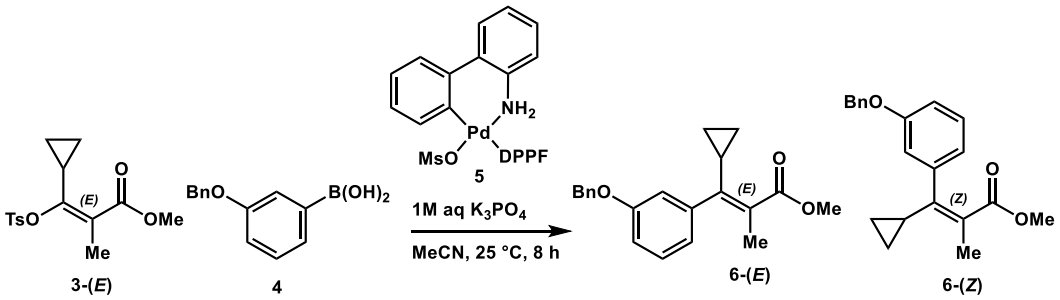
*Synthesis of (E)-Methyl 3-cyclopropyl-2-methyl-3-(tosyloxy)acrylate **3-(E)***



To a 500 ml round bottomed flask equipped with a magnetic stir bar under N_2 atmosphere was charged methyl 3-cyclopropyl-3-oxopropanoate **1** (39.2 g, 276 mmol), MeI (17.2 ml, 276 mmol) and K_2CO_3 (57.2 g, 414 mmol). The mixture was agitated at 25 °C for 16 h. The reaction mixture was diluted with Et_2O (100 ml) and filtered. The solids were rinsed with Et_2O (100 ml) and the filtrate was concentrated to a crude oil. The crude oil was purified by normal phase column chromatography to give methyl 3-cyclopropyl-2-methyl-3-oxopropanoate **2**[1] in 79% yield.

To an oven-dried 1000 ml round bottomed flask equipped with a magnetic stir bar under N_2 atmosphere was added methyl 3-cyclopropyl-2-methyl-3-oxopropanoate **2** (10.0 g, 64.0 mmol) and THF (100 ml). The mixture was cooled to -78 °C and 1M NaHMDS solution (96.0 ml, 96.0 mmol) was added over 30 min, maintaining the internal temperature below -60 °C, followed by a THF rinse (50 ml). The mixture was aged for 1 h at -78 °C and Ts_2O (31.3 g, 96.0 mmol) in THF (200 ml) was added over 1 h, maintaining the internal temperature below -60 °C. The mixture was aged for 20 min at -78 °C, then warmed to 25 °C and aged for 2 h. A thick slurry formed. The reaction was quenched with 300 ml of 0.5M $NaHCO_3$ solution and the phases were separated. The aqueous layer was back-extracted twice with 100 ml of EtOAc. The organic layers were combined and washed with 200 ml of saturated NaCl solution, dried over $MgSO_4$ and filtered. Upon concentration to an oil, the crude product was purified by normal phase column chromatography to give (E)-methyl 3-cyclopropyl-2-methyl-3-(tosyloxy)acrylate **3-(*E*)**[1] in 30% yield and (Z)-methyl 3-cyclopropyl-2-methyl-3-(tosyloxy)acrylate **3-(*Z*)**[1] in 54% yield.

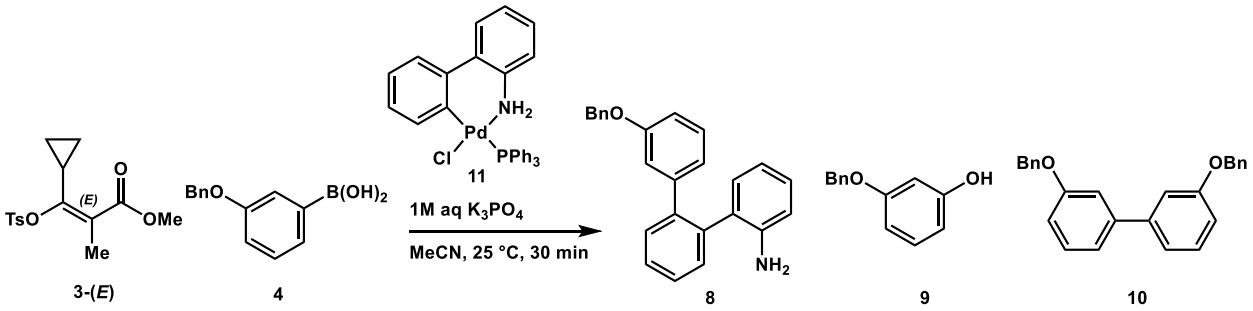
*Synthesis of (E)-Methyl 3-(3-(benzyloxy)phenyl)-3-cyclopropyl-2-methylacrylate **6-(E)***



To a 40 ml reaction vial equipped with a magnetic stir bar under N_2 atmosphere was charged (E)-methyl 3-cyclopropyl-2-methyl-3-(tosyloxy)acrylate **3-(*E*)** (1.00 g, 3.22 mmol), (3-(benzyloxy)phenyl)boronic acid **4** (0.808 g, 3.54 mmol), and palladium precatalyst **5** (0.298 g, 0.322 mmol). Acetonitrile (15 ml) and 1M aqueous K_3PO_4 solution (9.67 ml, 9.67 mmol) were added and the reaction agitated at 25 °C for 8 h. To the reaction was added 10 ml of saturated NH_4Cl quench and organic layer was separated from the aqueous layer. The aqueous layer was back-extracted twice with 10 ml of ethyl acetate. The combined organic layers were washed with 10 ml of saturated NaCl solution, dried over $MgSO_4$ and filtered. Upon concentration to an oil, the crude product was purified by normal phase column chromatography to give (*E*)-methyl 3-(3-(benzyloxy)phenyl)-3-cyclopropyl-2-methylacrylate **6-(*E*)** in 52% yield and (*Z*)-methyl 3-(3-(benzyloxy)phenyl)-3-cyclopropyl-2-methylacrylate **6-(*Z*)**[1] in 33% yield.

(*E*)-Methyl 3-(3-(benzyloxy)phenyl)-3-cyclopropyl-2-methylacrylate **6-(*E*)** ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.45 – 7.42 (m, 2H), 7.40 – 7.36 (m, 2H), 7.33 – 7.27 (m, 2H), 6.95 (dd, *J* = 8.3, 1.9 Hz, 1H), 6.57 – 6.54 (m, 1H), 6.52 (d, *J* = 7.5 Hz, 1H), 5.11 (s, 2H), 3.72 (s, 3H), 2.56 – 2.51 (m, 1H), 1.49 (s, 3H), 0.66 – 0.53 (m, 2H), 0.20 – 0.08 (m, 2H).
^{13}C NMR (126 MHz, DMSO-d_6) δ 169.85, 158.44, 150.78, 138.69, 137.44, 129.84, 128.87, 128.26, 128.16, 124.33, 121.09, 115.03, 114.36, 69.64, 51.90, 18.21, 14.70, 5.64.

*Synthesis of 3''-(benzyloxy)-[1,1':2',1''-terphenyl]-2-amine **8**, 3-(benzyloxy)phenol **9** and 3,3'-bis(benzyloxy)-1,1'-biphenyl **10***



To a 40 ml reaction vial equipped with a magnetic stir bar under N_2 atmosphere was charged (E)-methyl 3-cyclopropyl-2-methyl-3-(tosyloxy)acrylate **3-(*E*)** (200 mg, 0.644 mmol), (3-(benzyloxy)phenyl)boronic acid **4** (162 mg, 0.709 mmol) and palladium precatalyst **11** (18.4 mg, 0.032 mmol). Acetonitrile (5 ml) and 1M aqueous K_3PO_4 solution (1.93 ml, 1.93 mmol) were added and the reaction was agitated at 25 °C for 30 min. To the reaction was added 10 ml of saturated NH_4Cl quench and organic layer was separated from the aqueous layer. The aqueous layer was back-extracted twice with 10 ml of ethyl acetate. The combined organic layers were washed with 10 ml of saturated NaCl solution, dried over $MgSO_4$ and filtered. Upon concentration to an oil, the crude product was purified by reverse phase HPLC to give 3''-(benzyloxy)-[1,1':2',1''-

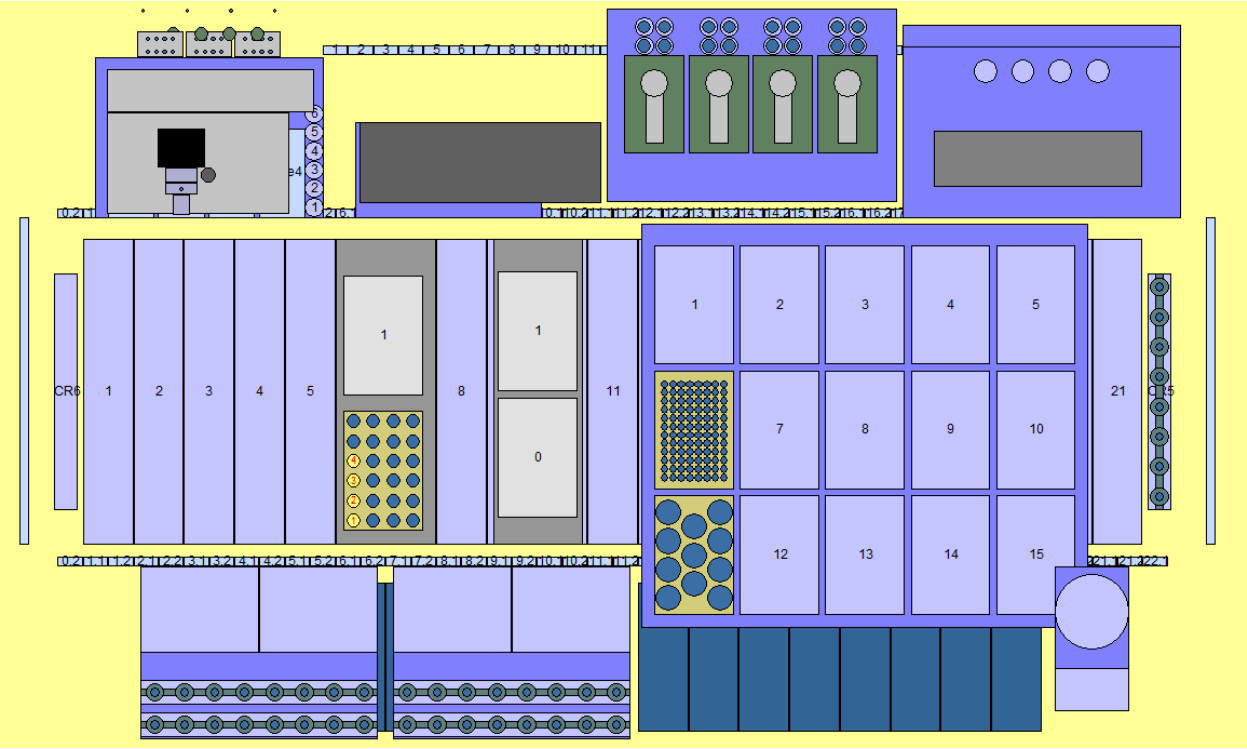
terphenyl]-2-amine **8** in 2% isolated yield, 3-(benzyloxy)phenol **9** in 7% isolated yield and 3,3'-bis(benzyloxy)-1,1'-biphenyl **10** in 4% isolated yield.

*3''-(benzyloxy)-[1,1':2',1''-terphenyl]-2-amine **8*** ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.49 – 7.41 (m, 3H), 7.40 – 7.34 (m, 4H), 7.34 – 7.27 (m, 2H), 7.13 (t, *J* = 7.8 Hz, 1H), 6.99 – 6.93 (m, 1H), 6.86 – 6.78 (m, 3H), 6.70 (dd, *J* = 7.5, 1.4 Hz, 1H), 6.64 (dd, *J* = 8.0, 0.9 Hz, 1H), 6.48 (td, *J* = 7.4, 1.0 Hz, 1H), 4.86 (s, 2H), 4.45 (s, 2H).
^{13}C NMR (126 MHz, DMSO-d_6) δ 158.14, 145.65, 142.71, 141.06, 138.19, 137.48, 131.42, 130.93, 130.57, 129.29, 128.87, 128.26, 128.23, 128.17, 128.03, 126.44, 121.81, 116.65, 115.62, 115.03, 113.96, 69.48.

*3-(benzyloxy)phenol **9*** ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.39 (s, 1H), 7.46 – 7.36 (m, 4H), 7.33 (d, *J* = 7.2 Hz, 1H), 7.05 (t, *J* = 8.1 Hz, 1H), 6.44 (dd, *J* = 8.2, 1.8 Hz, 1H), 6.39 (t, *J* = 2.3 Hz, 1H), 6.39 – 6.33 (m, 1H), 5.03 (s, 2H).
^{13}C NMR (126 MHz, DMSO-d_6) δ 160.03, 159.02, 137.72, 130.31, 128.87, 128.20, 128.05, 108.49, 105.94, 102.55, 69.46.

*3,3'-bis(benzyloxy)-1,1'-biphenyl **10*** ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.51 – 7.47 (m, 4H), 7.44 – 7.39 (m, 4H), 7.39 – 7.32 (m, 4H), 7.28 (t, *J* = 2.0 Hz, 2H), 7.24 (d, *J* = 7.7 Hz, 2H), 7.02 (dd, *J* = 8.1, 2.1 Hz, 2H), 5.19 (s, 4H).
^{13}C NMR (126 MHz, DMSO-d_6) δ 159.28, 142.00, 137.62, 130.42, 128.91, 128.29, 128.19, 119.78, 114.61, 113.60, 69.73.

2.3. Sample Chemspeed Autosuite Protocol for Automated Kinetic Profiling



1	Transfer Volumetrically	Transfer liquid from valve ports C to Waste 1 with Needle Head #1		02 min 16 sec
2	Heat / Cool	Thermostat ON on zone Reactions		15 min 00 sec
3	Stir	Agitation ON on zone Reactions		02 sec
4	HPLC Injection	Execute Once	Reaction Profiling	00 sec
1	Macro Task	Execute Once		00 sec
1	Transfer Volumetrically	Transfer liquid from Initiator to variable 'One_reaction' with Needle		00 sec
2	Macro Task	Execute Once		00 sec
1	Transfer Volumetrically	Transfer liquid from variable 'One_reaction' to variable 'one_sampl		00 sec
2	Transfer Volumetrically	Transfer liquid from variable 'one_sample' to variable 'one_sample'		00 sec
3	Transfer Volumetrically	Transfer liquid from variable 'one_sample' to Injection valve 2 Loac		00 sec
4	Wait	Waiting for 2 seconds		02 sec
5	Switch Contact	Switch electrical contact 1 on		00 sec
6	Wait	Waiting for 2 seconds		02 sec
7	Switch Contact	Switch electrical contact 1 off		00 sec
8	Transfer Volumetrically	Transfer liquid from Port C 1 to Injection valve 2 Inject with Needle		43 sec
9	Wait	Waiting for 2:45 minutes		02 min 45 sec

Name: HPLC Injection

Variable Name	Array	Initial Value	Unit/Type
injection_vol	☐	50	ul
valve_rinse_vol	☐	1	ml
needle_rinse_vol	☐	1	ml
spl_vol	☐	20	ul
mix_vol	☐	200	ul
mix_airgap_vol	☐	200	ul
initiator_vol	☐	375	ul

Loop Variable: loop Fragment Variable: fragment

◉ Execute 1 $ Times

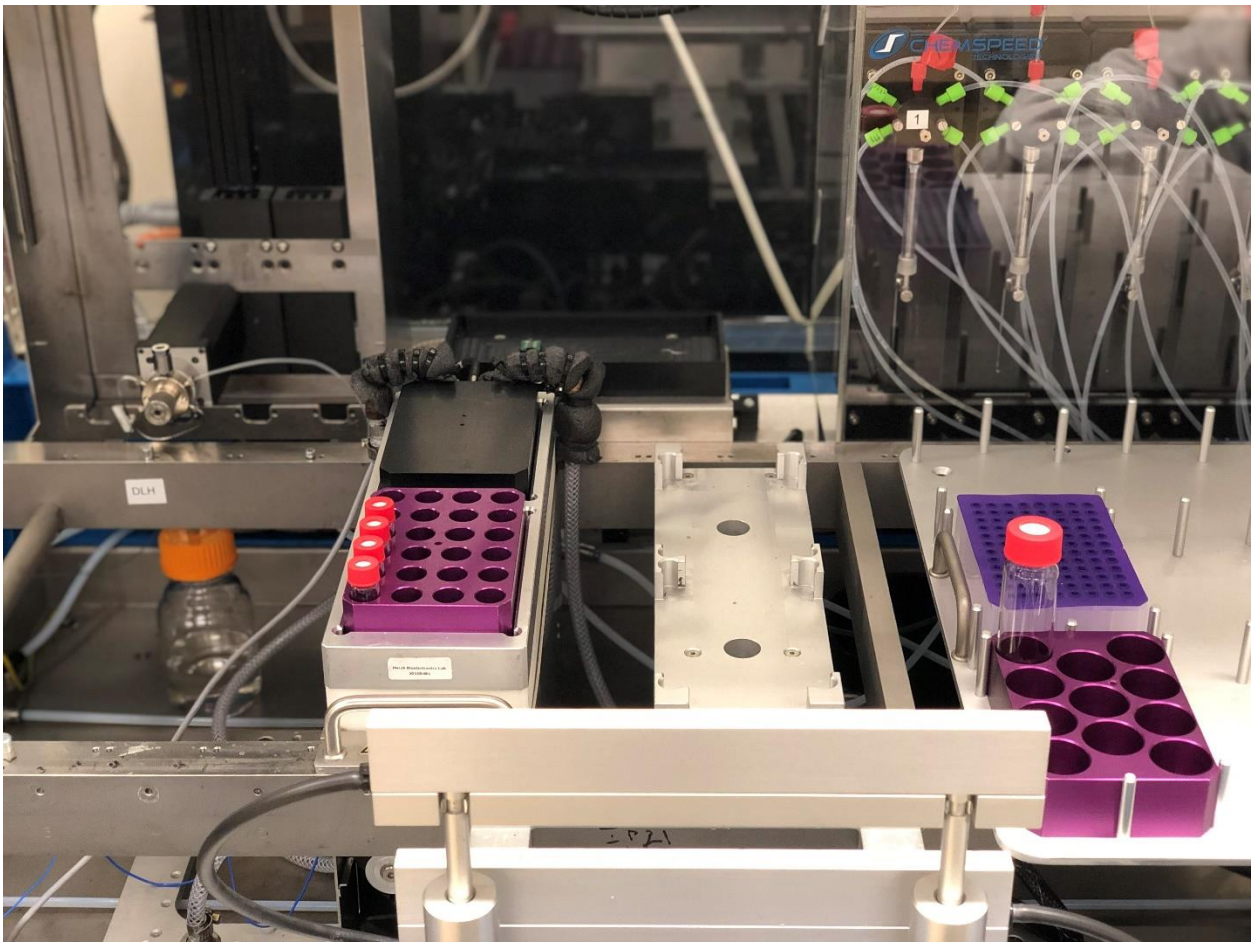
○ Execute if condition

○ Execute while condition

○ Batch ◉ Sequential

'Sequential Zone' Variable Name	Value	Fragment Size
One_reaction	Reactions	1
Rxtn_samples	LC Samples	24

2.4. Picture of Chemspeed Robot Deck



3. Characterization of New Compounds

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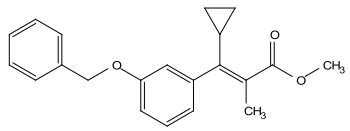

6-(E)

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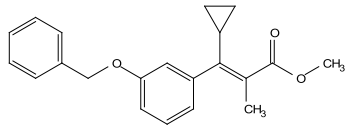

6-(E)

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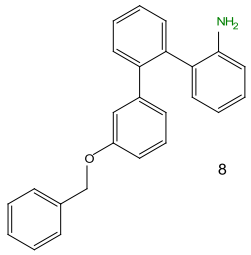

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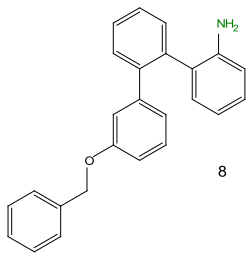

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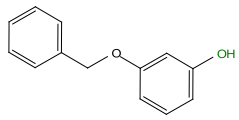

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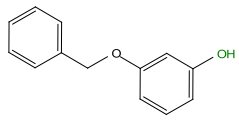

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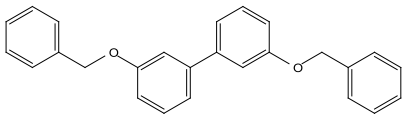

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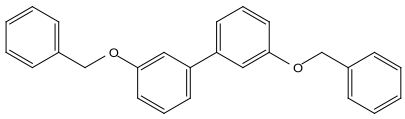

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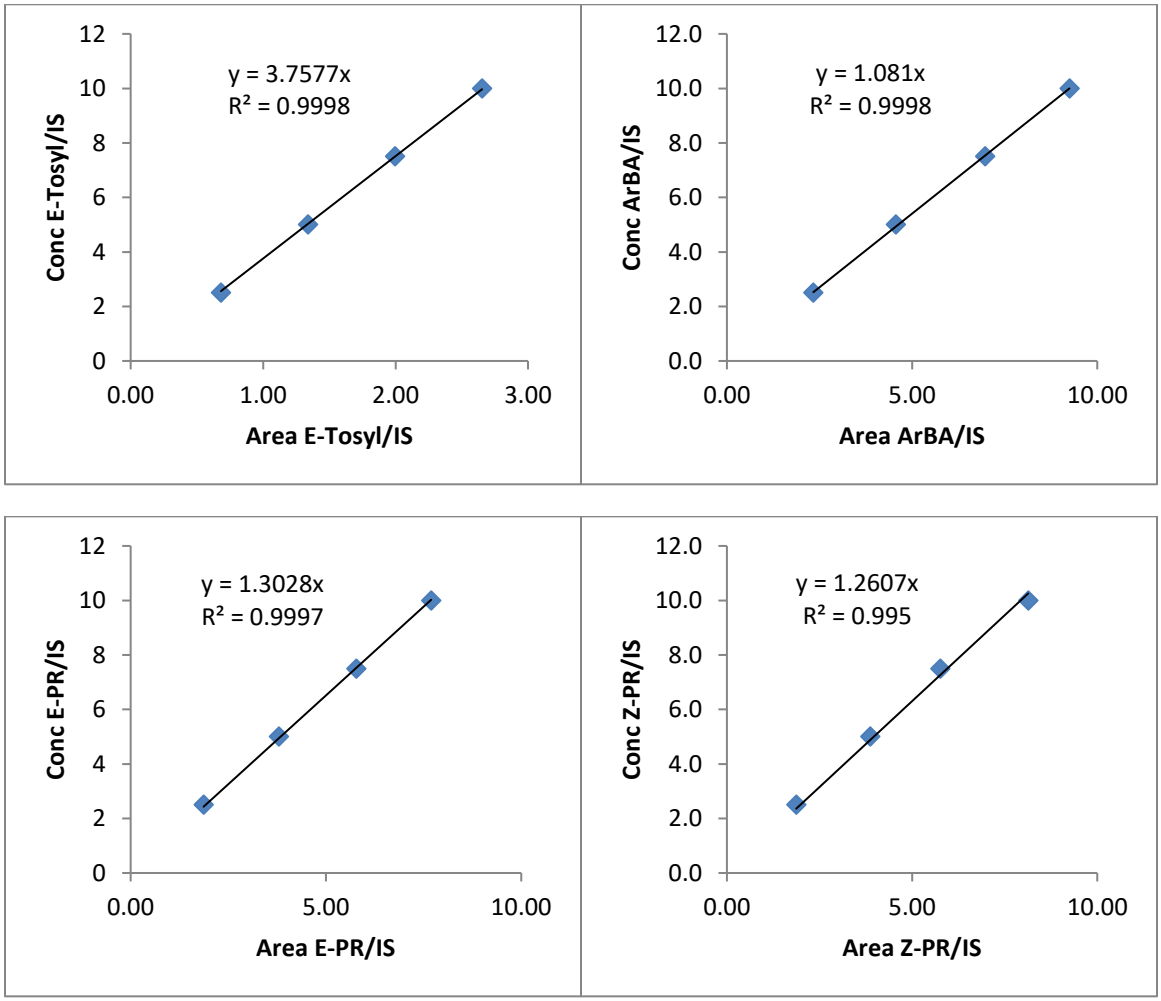
4. Calibration Curves

Four mixtures of varying molar concentration ratios of target compound to 1,3,5-trimethoxybenzene were prepared and injected on the online HPLC system. These molar ratios were then plotted against the area count ratios of target compound to 1,3,5-trimethoxybenzene. Upon linear regression with the intercept set at zero, the response factor for each target compound was determined and used to calculate the concentration values presented in Figures 2, 4 and 5 of the main manuscript.



[i] See section 1.3. Analytical Methods for preparation of pH 3.5 ammonium formate buffer.

[1] These compounds were previously characterized in M. Christensen, A. Nolting, M. Shevlin, M. Weisel, P. E. Maligres, J. Lee, R. K. Orr, C. W. Plummer, M. T. Tudge, L.-C. Campeau and R. T. Ruck, *J. Org. Chem.*, 2016, **81**, 824–830.