Definitive screening designs for kinetic models in flow

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Contents

1   Experimental

1.1   General preparation of feed solutions

The input feed solutions were (1) 2,4-dichloropyrimidine (2,4-DCP) **1** and the aluminium trichloride ($AlCl_3$) pre-mixed in acetonitrile and (2) 1-methylindole (Me-Ind) **2** dissolved in acetonitrile. The input solutions were prepared within a glove box and sealed with a septum prior to removal for the flow experiments. The solutions were used straightaway in the flow reaction. The input solutions were prepared individually for each DSD experiment to develop an understanding regarding the sensitivity of the response to input solution variation.

Preparation of feed solutions for centre point experiment

Preparation of input solution A. A 30 mL feed solution was prepared by dissolving 2,4-DCP **1** (4.00 g, 0.0270 mol, 1 equiv.) in anhydrous acetonitrile. Subsequently, $AlCl_3$ (3.94 g, 0.0300 mol, 1.10 equiv.) was added portionwise to the solution. On addition of $AlCl_3$ an exotherm was observed and some white fumes observed in the headspace. The mixture was stirred over 10 min to ensure that all solids had dissolved.

Preparation of input solution B. A 30 mL feed solution was prepared by dissolving 1-methylindole (Me-Ind) **2** (3.72 mL, 0.0298 mol, 1.11 equiv.) in anhydrous acetonitrile.

The solutions were used straightaway in the flow reaction at 100 °C and a concentration-residence time profile was collected by varying the pump flow rate appropriately.

1.2   Flow experiments

The flow experiments were conducted using a Vapourtec E-series (Figure 1). The two feeds solutions were delivered using two peristaltic pumps. The pumps were calibrated prior to use. The two streams were mixed *via* a tee-piece at ambient temperature prior to entering the reactor. A standard Vapourtec manifold heating reactor fitted with a PFA coil (internal diameter 1 mm) was used as the reactor. The high heat transfer provided consistent temperature across the tubing (±1 °C) measured by using a thermocouple inserted into the glass manifold holding the PFA coil. The reaction was homogeneous across the range of conditions studied. The volumetric flow rate was changed to give measurements at four different residence times for each DSD experiment. The tee-piece was cleaned regularly to prevent blockages caused by the formation of $Al(OH)_3$, observed as a white solid. The reactor was washed with a water/isopropanol mixture to avoid clogging. The conditions used for each experiment can be seen in Table 1. Four residence time experiments were conducted for each DSD experiment giving a total of ten profiles and 40 experiments. The reactant concentrations and residence time were corrected for the effects of thermal expansion within the flow reactor. The residence time (min) was defined as the total coil reactor volume (mL) divided by the corrected flow rate (mL/min) and did not

include the tee-piece volume. The flow rate was corrected using the formula reported by Mozharov *et al* (equation 1).[1]

$$F_{eff} = 1 + \alpha_v(T_1 - T_0)F \quad (Equation\ 1)$$

Where: F_{eff} = effective flow rate (mL/min), α_v = volumetric coefficient of thermal expansion at 1 bar pressure (1/°C), T_1 = reaction temperature (°C), T_0 = temperature prior to entering reactor (T_0 = 20 °C) and F = volumetric flow rate (mL/min).

1.3 Validation experiment isolation protocol

3-(2-Chloropyrimidin-4-yl)-1-methylindole (3). The reaction was conducted using the optimal conditions described in the manuscript. [The yield was calculated based on the purification of a fraction collected based on 2,4-dichloropyrimidine (**1**) (~1.00 g, 6.71 mmol) being processed] The reaction afforded 3-(2-chloropyrimidin-4-yl)-1-methylindole (**3**) in 80% UPLC yield (82% predicted). The compound was then purified by using a protocol reported previously in the literature.[2] The collected reaction mixture was added dropwise to vigorously stirring water (50 mL) over 5 min. Upon complete addition the mixture was stirred for 30 min, filtered and the solid washed with water (50 mL). The crude product was purified by flash silica chromatography, eluting with DCM. Pure fractions were evaporated under reduced pressure to afford pure 3-(2-chloropyrimidin-4-yl)-1-methylindole (**3**) (1.13 g, 4.63 mmol 69%) as an off-white solid. ^{1}H NMR (300 MHz, DMSO) δ3.90 (3H, s), 7.30 (2H, m), 7.54−7.6 (1H, m), 7.82 (1H, d), 8.38−8.44 (1H, m), 8.49 (1H, s), 8.53 (1H, d). The data were consistent with literature data.[2]

2   UPLC Analysis

The work-up and analysis protocol was repeated twice to ensure reproducibility in the post-reaction and analysis stages. Neat reaction samples were collected in dram vials and weighed. Dichloromethane (1 mL) was added followed by water (2 mL). The complexes were liberated by using this protocol. An aliquot of the dichloromethane layer (100 µL) was then taken and then pipetted into a new dram vial and then weighed. Acetonitrile (1 mL) was then added and the sample weighed. The reactions were subsequently monitored offline by UPLC. All the reported values are nonisolated and based on normalisation using the relative response factors. Response factors were measured for dichloropyrimidine **1**, 1-methylindole **2**, desired product **3**, isomer **4** and *bis*-indole **5**. There was an average mass balance loss of 4% with respect to the pyrimidine reaction components. This loss could be from the reaction and/or work-up stage.

All the reactions within this paper were analysed using the same UPLC method. Processed reaction sample was dissolved in 10% aqueous acetonitrile. UPLC quantitative analysis was performed on a Waters Acquity using a BEH phenyl (100 mm length, internal diameter 2.1 mm and 1.7 μL particle size) operating at 40 °C throughout. Mobile phase A was water and mobile phase B was acetonitrile, and each contained 0.03% TFA. Mobile phase B was ramped from 5% to 95% over 15 min and then returned to 5% at 15.1 min. Mobile phase B was kept at 5% until 17 min. The total pump flow rate was 0.6 mL/min. Total UPLC run time was 17 min. An example UPLC chromatogram is shown in Figure S1.
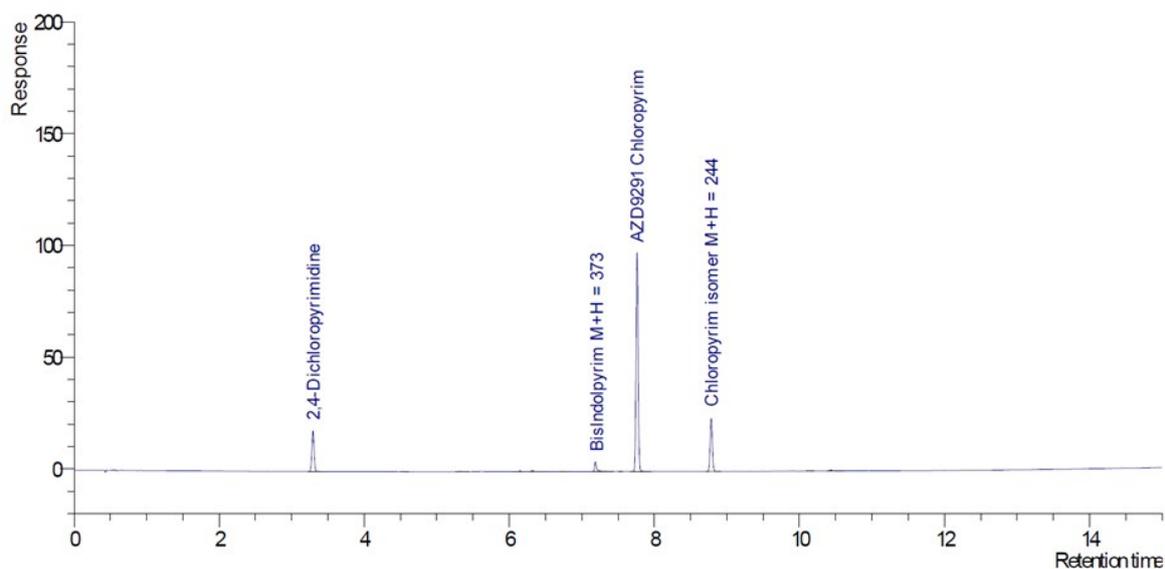

Figure S1. Typical UPLC chromatogram for the reaction.

2.1 Raw data (Table S1)

Exp	input T (°C)	input DCP (M)	input Indole (M)	input AlCl$_3$ (M)	input t_{res} (min)	calc tau	calc DCP (M)	response Product (M)	response Isomer (M)	response Bis (M)
1	100	0.39	0.44	0.43	2.22	8.27	0.2704	0.0923	0.0200	0.0014
2	100	0.39	0.44	0.43	2.94	10.92	0.2443	0.1106	0.0248	0.0017
3	100	0.39	0.44	0.43	4.45	16.54	0.2055	0.1452	0.0346	0.0029
4	100	0.39	0.44	0.43	8.89	33.09	0.0918	0.2310	0.0398	0.0066
5	120	0.38	0.54	0.57	2.13	7.91	0.1100	0.2300	0.0333	0.0093
6	120	0.38	0.54	0.57	2.81	10.44	0.0670	0.2600	0.0381	0.0129
7	120	0.38	0.54	0.57	4.25	15.82	0.0231	0.2784	0.0469	0.0188
8	120	0.38	0.54	0.57	8.50	31.63	0.0029	0.2866	0.0451	0.0358
9	80	0.40	0.32	0.28	2.30	8.54	0.3678	0.0181	0.0112	0.0000
10	80	0.40	0.32	0.28	3.03	11.27	0.3532	0.0203	0.0129	0.0000
11	80	0.40	0.32	0.28	4.59	17.08	0.3498	0.0283	0.0150	0.0000
12	80	0.40	0.32	0.28	9.18	34.16	0.2938	0.0547	0.0260	0.0000
13	120	0.34	0.37	0.24	2.13	7.91	0.1702	0.1339	0.0244	0.0050
14	120	0.34	0.37	0.24	2.81	10.44	0.1549	0.1451	0.0305	0.0064
15	120	0.34	0.37	0.24	4.25	15.82	0.1316	0.1614	0.0320	0.0085
16	120	0.34	0.37	0.24	8.50	31.63	0.0966	0.1705	0.0309	0.0162
17	80	0.47	0.52	0.71	2.30	8.54	0.4300	0.0454	0.0256	0.0003
18	80	0.47	0.52	0.71	3.03	11.27	0.4100	0.0506	0.0256	0.0003
19	80	0.47	0.52	0.71	4.59	17.08	0.3626	0.0575	0.0275	0.0005
20	80	0.47	0.52	0.71	9.18	34.16	0.3271	0.0826	0.0307	0.0014
21	80	0.33	0.37	0.37	2.30	8.54	0.2934	0.0211	0.0090	0.0001
22	80	0.33	0.37	0.37	3.03	11.27	0.2815	0.0294	0.0122	0.0001
23	80	0.33	0.37	0.37	4.59	17.08	0.2759	0.0393	0.0133	0.0002
24	80	0.33	0.37	0.37	9.18	34.16	0.2453	0.0524	0.0214	0.0005
25	120	0.48	0.39	0.53	2.13	7.91	0.1852	0.2231	0.0555	0.0057
26	120	0.48	0.39	0.53	2.81	10.44	0.1625	0.2447	0.0562	0.0057
27	120	0.48	0.39	0.53	4.25	15.82	0.1289	0.2724	0.0570	0.0061
28	120	0.48	0.39	0.53	8.50	31.63	0.1275	0.2932	0.0570	0.0058
29	100	0.47	0.66	0.33	2.22	8.27	0.3223	0.1059	0.0259	0.0009
30	100	0.47	0.66	0.33	2.94	10.92	0.2911	0.1443	0.0314	0.0010
31	100	0.47	0.66	0.33	4.45	16.54	0.2148	0.1768	0.0432	0.0017
32	100	0.47	0.66	0.33	8.89	33.09	0.1173	0.2774	0.0639	0.0031
33	100	0.34	0.27	0.50	2.22	8.27	0.2534	0.0573	0.0099	0.0010
34	100	0.34	0.27	0.50	2.94	10.92	0.2463	0.0661	0.0108	0.0014
35	100	0.34	0.27	0.50	4.45	16.54	0.2271	0.0918	0.0152	0.0027
36	100	0.34	0.27	0.50	8.89	33.09	0.1394	0.1353	0.0253	0.0062

3 Model Determination

To demonstrate the potential to utilise the data for the consideration of different kinetic models, we trialled fitting different model structures using DynoChem software (Scale-up Systems). The experimental data was used to compare three different proposed kinetic models. The different motifs (model structures) proposed to represent the reaction system were: (i) third

order rate-limiting with first order with respect to all the reaction components (model 1); (ii) second order rate determining with first order with respect to pyrimidine reacting species and 1-methylindole, and 0 order with respect to $AlCl_3$ (model 2); (iii) a fast reaction between pyrimidine reacting species and $AlCl_3$ (set at a pre-defined value for rate constant) and a subsequent second order rate-limiting step with first order respect to the pyrimidine reacting species and 1-methylindole **2** (model 3).

Based on different statistical analysis techniques, the model structure displaying second order kinetics, first order with respect to the Me-Ind **2** and first order in the pyrimidine complex, provided the best fit (model 3) (Table S2), which is in line with expectations based on existing kinetic studies for similar systems. The model selection criteria (MSC) is an analysis technique used to compare different models with the model with the higher MSC being the better model.

Table S2. Comparison of model fit for different kinetic motifs for the reaction steps.

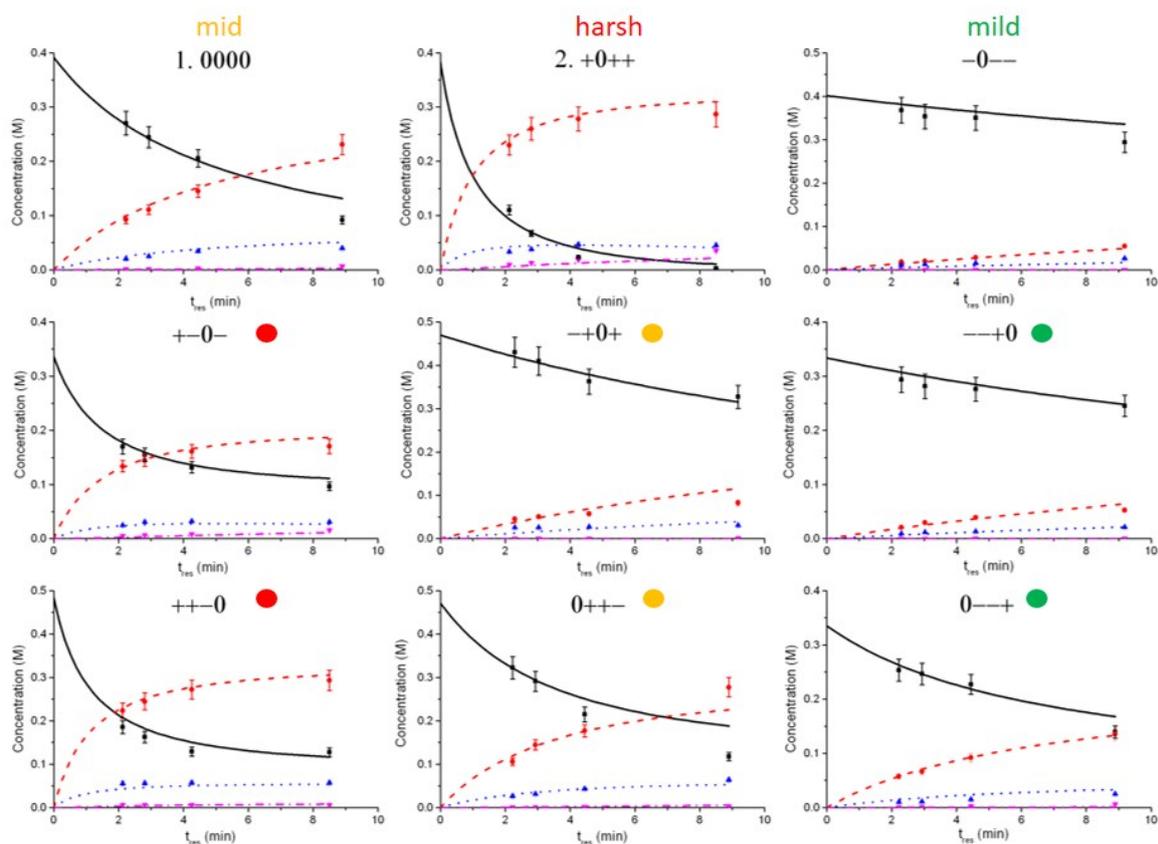| Statistics	Model 1	Model 2	Model 3 (discussed in manuscript)
Sum of squares	3.67	5.17	6.13
Variance	0.0307	0.0377	0.0449
Standard deviation	0.175	0.194	0.212
R^2	0.813	0.89	0.861
Goodness of fit	0.482	0.484	0.484
F-statistic	987	1368	3180
F-crit	2.02	2.07	2.16
Model selection criteria	3.39	3.48	4.46

4 Model fitted graphs with colour

Figure S2. Concentration-time profiles from simultaneous parameter fitting, points = experiments ■ 2,4-DCP **1**, ● Product **3**, ● Isomer **4**, ▼ *bis*-**5**, lines = model predicted using Table 2 kinetic parameter estimates. See Table S1 for raw experimental data.

5 Isothermal Fitting

Initially, the rate constants were fitted to the two centre point profiles, enabling the fit for the rate constants to be decoupled from the activation energies for the pathways, therefore minimising the correlation between rate constant and activation energy for each step. The initial fit for the rate constants is shown in Table S3, the ratio of desired product to isomer formation is 4.5 at T_{ref} = 100 °C. The model displayed a good fit, with R^2 = 0.995.

Table S3. Optimal rate constant parameter estimates and uncertainties from T = 100 °C profile (centre point experiment)

Kinetic parameter	Value ± SE ($M^{-1} s^{-1}$)
k_2	6.20 ± 0.39
k_3	1.40 ± 0.07
k_4	1.90 ± 0.12

[a]Uncertainties given as ±1 standard deviation and in the same units as k. 95% confidence level, N_{exp} =4, R^2 = 0.995, σ = 0.0593.

6   Exploring a wide design space

The dimensionless time plot, see Figure S3, represents the relative reactivity of all the input conditions used in the experimental study, a higher number indicates higher reactivity. The plot shows that experimental data were collected from very mild to aggressive conditions, within the constraints of the equipment and at process relevant conditions, were studied thus allowing the kinetics for the reaction system to be fitted.
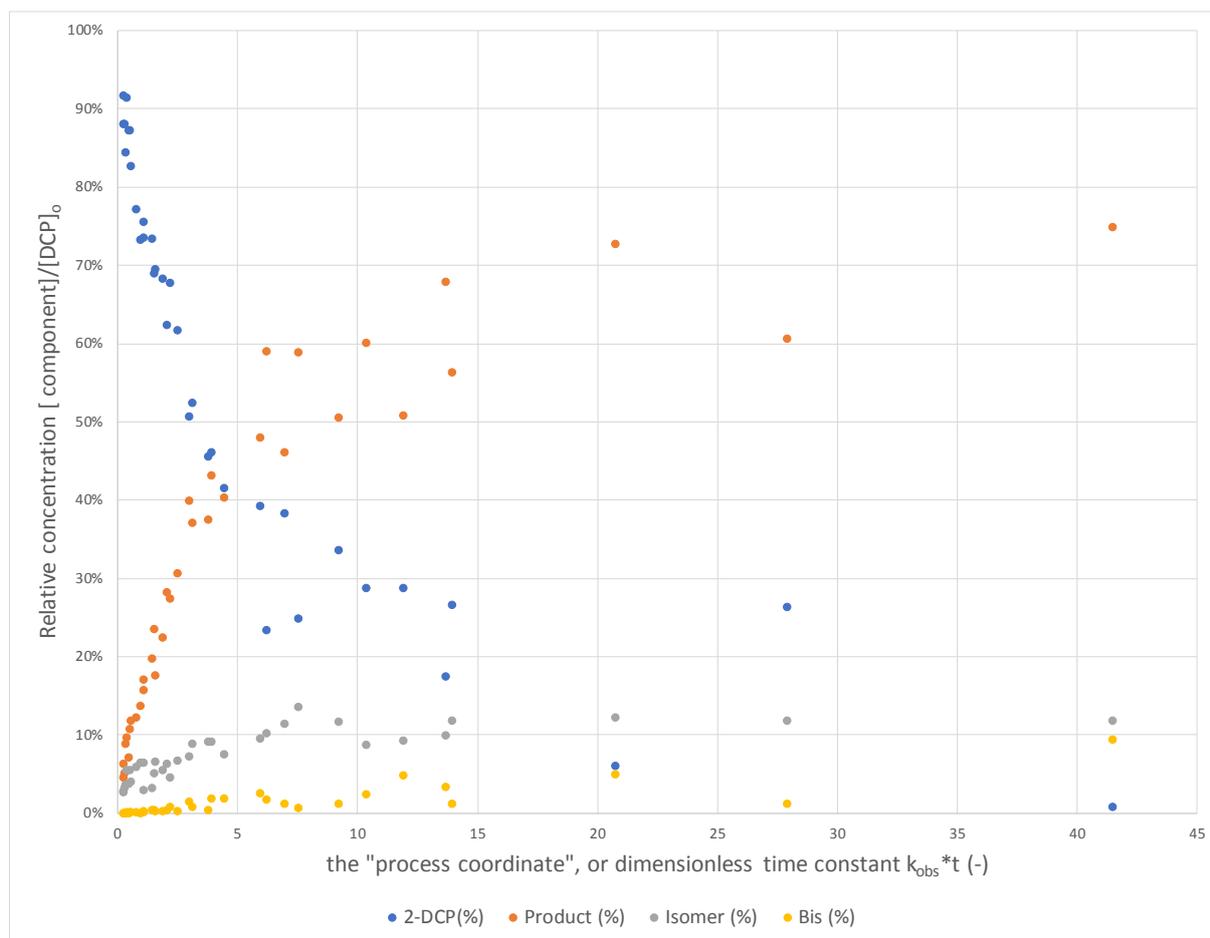

Figure S3. Dimensionless time plot to show the spread of responses relative to the dimensionless time constant.

7   Statistical Models

The experimental data was also fitted to generate polynomial equations for 2,4-DCP **1** conversion, desired product **3**, isomer **4** and *bis*-indole **5** yield. Statistical modelling was performed using MODDE (MODeling and DEsign) software. The models were fitted using multiple linear regression (MLR). The models included main effects, second order effects and interaction effects for each input parameter with time (Equation S1).

$$y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_4x_4 + b_5x_5 + b_{11}x^2 + b_{22}x^2 + b_{33}^2x^2 + b_{44}^2x^2 + b_{55}^2x$$
$$b_{35}x_2x_3 + b_{45}x_1x_4 \quad (Equation\ S1)$$

b_0 was the intercept, b_1, b_2, b_3, b_4, b_5, b_{15}, b_{25}, b_{35}, b_{45}, b_{11}^2, b_{22}^2, b_{33}^2, b_{44}^2 and b_{55}^2 were the coefficient terms, x_1 was 2,4-DCP **1** concentration, x_2 was Me-Ind **3** equivalents, x_3 was AlCl$_3$ equivalents, x_4 was temperature and x_5 was residence time. The non-significant coefficient terms were removed from the models for each response and the significant coefficient terms (different from the noise) are shown in Figure S4. The summary statistics for the models is shown in Table S5.
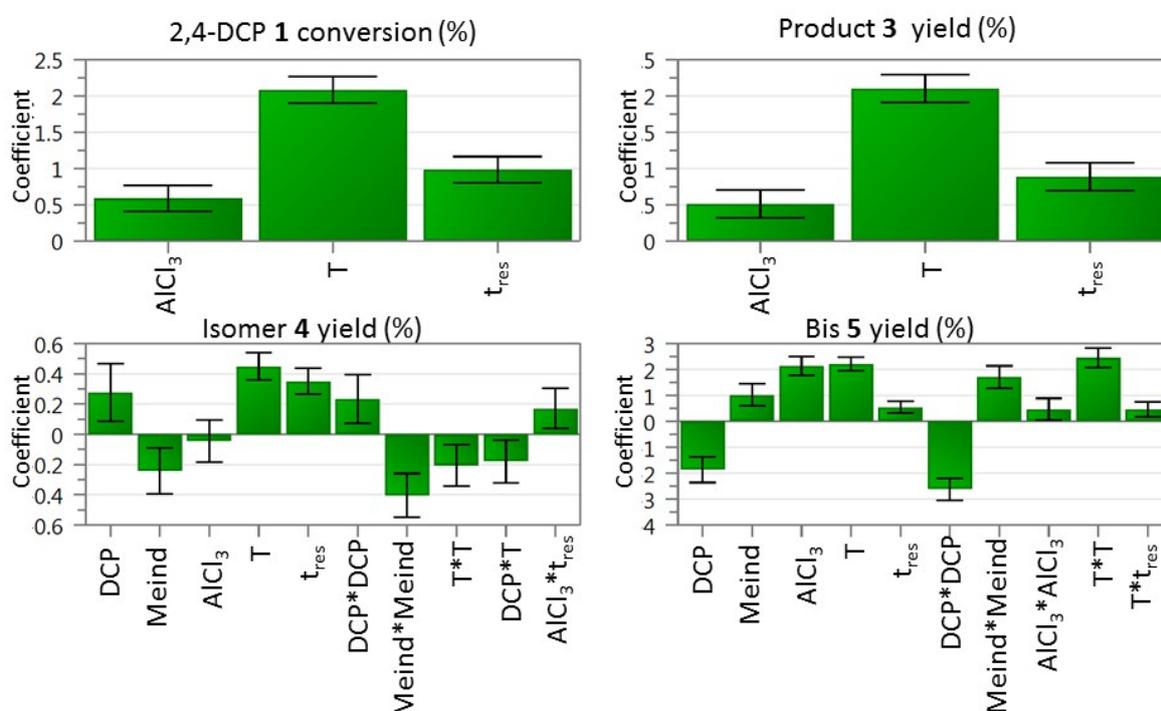

Figure S4. Coefficient plots for 2,4-DCP **1** conversion, desired product **3**, isomer **4** and *bis*-indole **5** yield after removing non-significant coefficient terms.

Table S5. Summary statistics from the statistical polynomial models.

Statistics	2,4-DCP **1** conversion	Product **3** yield	Isomer **4** yield	Bis-indole **5** yield
R^2	0.952	0.945	0.911	0.979
R^2 adjusted	0.948	0.940	0.881	0.972
Q^2	0.938	0.920	0.782	0.953

8   References

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