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Kinetic Resolution

Kinetic Resolution of Oxazinones: Rational Exploration of Chemical Space through the Design of Experiments

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Abstract: The organocatalytic kinetic resolution of 4-substituted oxazinones has been optimised (selectivity factor *S* up to 98, chiral oxazinone *ee* values up to 99.6% (**1a–g**) and product *ee* values up to 90% (**3a–g**)) in a rational way by applying the Design of Experiments (DoE) approach.

Introduction

Kinetic resolution is one of the classical methods for the synthesis of β-amino acids, which are key structural elements for the preparation of natural products, pharmaceuticals, and peptides.[1]

In 1996 the IUPAC defined kinetic resolution[2] (KR) as "the achievement of partial or complete resolution by virtue of unequal reaction rates of the enantiomers in a racemate by means of a chiral agent (catalyst, reagent, solvent, etc.)".[3] The outcome of this process is based on the different reaction rates of two diastereomorphic transition states. In light of optimising the conversion and the enantiomeric excess (*ee*) of both the starting materials and the products, kinetic resolution is among the reactions that are most affected by experimental conditions. A number of variables can have an influence on kinetics, for example temperature, concentration of reagents, reaction time, type of solvent, and the type of catalyst and its loading. Therefore, to optimise a kinetic resolution reaction, a large number of parameters need to be assessed. Until recently, chemical space was explored mostly by using chemical intuition. Chemists, for example, design and synthesise different catalysts in the quest to find the best performing derivative, and optimise reaction conditions mostly by employing a trial-and-error approach. However, as in other fields of science, it is highly desirable to explore experimental space by means of a rational approach. Design of Experiments (DoE) provides a systematic tool with which to explore the chemical space in a rational way. This approach involves the application of a set of statistical and mathematical tools that have been known since the 1930s and extensively exploited in industry, but which is also starting to receive attention in academia.[4] The "classic" chemical space exploration follows the change-one-variable-at-a-time (OVAT) approach. This strategy assumes that the variables influencing the reaction do not interact with each other. However, especially in a kinetic resolution process, variables are actually likely to interact with each other. By using the traditional approach, finding the optimum conditions would require a large number of experiments, and if interactions are significant, optimum conditions may never be found. By applying DoE, it is possible, by varying all factors simultaneously over the set of experiments, to identify the most important variables to be controlled and with the right design, it may also be possible to study their interactions, to make models and predictions leading in a rational route to the real optimum conditions.[5] It should be stressed that DoE saves time towards optimisation by drastically reducing the number of experiments, but its main advantages are, rather, the rational exploration of the chemical space, which enables the identification of optimum conditions. By using a suitable design, it is also possible to develop empirical predictive models for systems, which would be unfeasible by any other approach. For example, the optimisation of reaction conditions by means of computational chemistry would require taking into account solvent and weak noncovalent interactions. These would introduce an error that is orders of magnitude above the 2 kcal mol^{-1} energy difference required for a reaction to proceed with approximately 90% *ee*.

The application of DoE and statistical methods for optimisation of asymmetric reactions within academia is gathering pace. Sigman applied surface response modelling to optimise ligands for metal-catalysed asymmetric allylation and asymmet-

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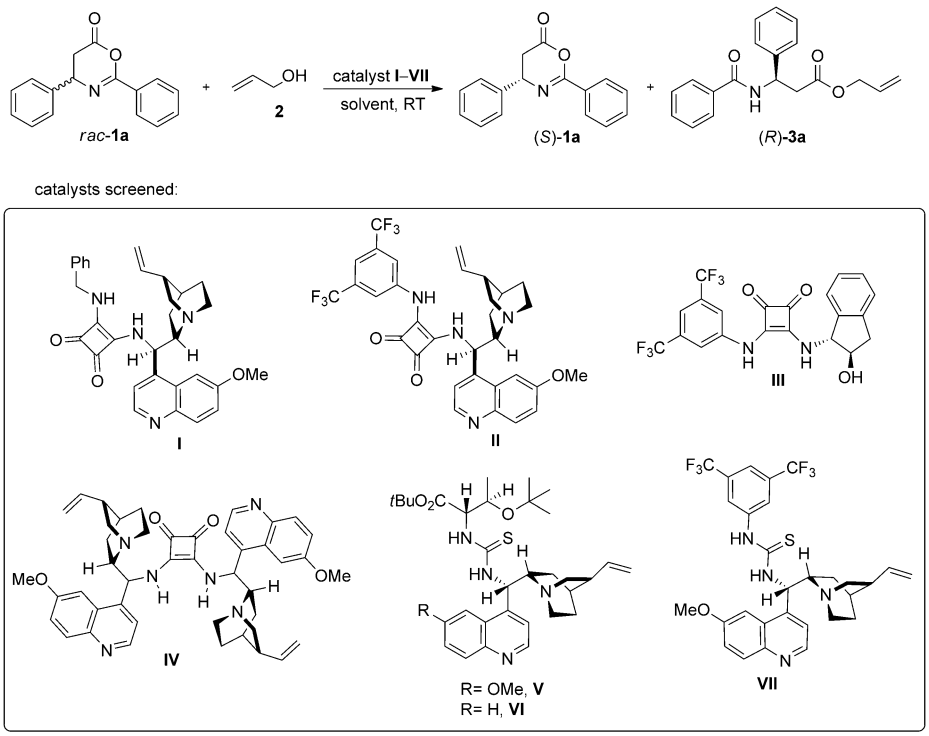
ric propargylation.[6] Recently, our research group applied DoE to optimise the reaction conditions for a challenging aza-Michael addition of imides mediated by multifunctional and multiple catalysis.[7] To the best of our knowledge, there is only one report to date on kinetic resolution and statistical optimisation. In 2011, Yadav et al. employed response surface methodology and Box–Behnken design to optimise a lipase-catalysed enantioselective resolution of (*R*,*S*)-2-pentanol.[8]

Results and Discussion

Pre-DoE experiments: preliminary catalyst screening

The asymmetric alcoholytic ring-opening of 4-substituted oxazinones is a known reaction, developed in 2005 by the group of Berkessel.[9] With the aim of exploring a new and broadly applicable strategy for reaction optimisation, we chose this transformation to demonstrate how the application of DoE could improve the previous results to a level that would not have been possible to achieve with any other approach. In general, beyond the synthetic importance of the investigated reaction, our aim was to show that this strategy could serve as a standard protocol for scientists dealing with similar issues.



Scheme 1. Alcoholytic ring-opening of 4-substituted oxazinone *rac*-**1a** in the presence of chiral organocatalysts **I**–**VII**.

At the outset of the study, it was useful to run some pre-DoE experiments, to choose suitable variable ranges. A control reaction on the model substrate, racemic 2,4-diphenyl-4,5-dihydro-1,3-oxazin-6-one (*rac*-**1a**), showed sluggish formation of the product *rac*-**3a**.[10] A preliminary catalyst screening was then performed to test chiral squaramide and thiourea catalysts **I**–**VII** (Scheme 1).[11] We anticipated that these compounds could be promising organocatalysts because bifunctional thioureas and squaramides proved to be highly effective catalysts for the dynamic kinetic resolution of azlactones[11d, 12] and for the kinetic resolution of oxazinones.[9] This screening was performed by employing *rac*-**1a** as substrate, allyl alcohol **2** (1 equiv) as nucleophile and 12.5% mol catalyst **I**–**VII** in anhydrous toluene as solvent.

To ensure reproducibility of the results, the reactions were run in a glove-box, as a precaution to prevent any possible ring-opening of the oxazinone by water; however, we will show that this was not necessary. In fact, modification of the previously reported procedure for the synthesis of the oxazinones and their purification by crystallisation afforded a compound that showed higher stability towards moisture in the solid form.[13] The results obtained are summarised in terms of enantiomeric excess of the starting material **1a**, enantiomeric excess of the product **3a** and conversion, in Table 1.

Table 1. Preliminary catalyst screening results.

Entry[a]	Cat.	*ee* **1a** [%][b]	*ee* **3a** [%][b]	Conv. [%][c]	Time [h]
1	**I**	−17[d]	−87[d]	16	17
2	**II**	−31[d]	−55[d]	36	17
3	**III**	*rac*	–	0	17
4	**IV**	88	88	50	2
5	**V**	62	82	42	2
6	**VI**	73	87	46	2
7	**VII**	77	74	51	2
8	**VII**	>99	65	>60	17

[a] Reaction conditions: *rac*-**1a** (0.1 mmol), **2** (1 equiv), catalyst **I**–**VII** (12.5 mol%), anhydrous toluene (0.85 mL). The reactions were performed in a glove-box. [b] The enantiomeric excess was determined by HPLC analysis on a chiral stationary phase. [c] The conversion was determined by HPLC analysis on a chiral stationary phase by comparison with the peak areas of stock solutions of the oxazinone *rac*-**1a** in toluene. Quantification was based on UV detection at $\lambda = 230$ nm. [d] Negative *ee* indicates the formation of the opposite enantiomer with respect to the one shown in Scheme 1.

Employing catalysts **I** and **II** the reaction was slow and, after 17 h, the enantiomeric excess of the starting material was only 17 and 31%, respectively (Table 1, entries 1 and 2). Squaramide **III** was not able to catalyse the reaction (entry 3). Promising results were instead obtained with the dimeric squaramide **IV** and with thioureas **V**–**VII** (entries 4–8). The pre-DoE experiments thus allowed identification of four potential catalysts for

the kinetic resolution of interest, selection of catalyst loading, and concentration range values to be used in the first DoE.

First DoE experiment: custom design

The first DoE was performed by applying a screening design to evaluate the main parameters and their influence on the reaction. The variables selected were: 1) type of catalyst (a categorical (discrete) variable with four levels: compounds **IV**–**VII**); 2) catalyst loading (a continuous variable in the range 5–20 mol%); 3) type of solvent (a categorical variable; five different solvents were chosen: toluene, dichloromethane, acetonitrile, *tert*-butyl methyl ether and ethyl acetate); 4) solution concentration (continuous variable in the range 0.05–0.5 M); 5) equivalents of allyl alcohol **2** (continuous variable in the range 0.5 to 1 equiv) and 6) temperature (continuous variable in the range 0–20 °C). The responses we desired to optimise were: enantiomeric excess of the starting material **1a**, enantiomeric excess of the allyl ester product **3a**, and conversion. A full factorial design (exploring all possible combinations of the six factors) would have required ($4\times2\times5\times2\times2\times2=320$ experiments. The use of a screening design allowed the number of experiments to be reduced. However, these types of design do not usually allow the study of all the interactions among parameters. We were especially interested in studying the interactions between solvent and catalyst. To generate a design capable of evaluating these interactions, we used the custom design function in the software JMP®.[14] As opposed to using standard textbook designs, this function is very useful to generate designs specifically tailored to the problem at hand. The design we generated required 24 experiments, the order of which was randomised. The results obtained were entered into the software to generate models and predictions for the three responses of interest. The sorted parameter estimates shown in Tables 2–4 classify the variables under study or their combinations in order of importance according to their influence on the reaction. The models obtained showed that the most important factors affecting the enantiomeric excess of the starting material and the conversion were the solution concentration, followed by the type of solvent, and temperature (see sorted parameters estimates, Tables 2 and 3). In all cases, toluene and dichloromethane seemed to be the best solvents to optimise these two responses. The type of catalyst did not appear to be important for either the enantiomeric excess of the starting material **1a** or for conversion, but it was the most important factor in terms of enantiomeric excess of the prod-

Table 2. Sorted parameter estimates across all time points for the enantiomeric excess of the starting material **1a**.

Term	Estimate[a]	Std Error	t Ratio[b]	Prob > |t|[c]
conc. *rac*-**1a** (0.05, 0.5 M)	17.137526	2.000436	8.57	<.0001*
solvent group (CH_2Cl_2/toluene)	10.951213	1.889159	5.80	<.0001*
temperature (0, 20 °C)	7.7067639	1.95869	3.93	0.002*
equivalents of **2** (0.5, 1 equiv)	7.071962	1.901479	3.72	0.0004*
catalyst load (5, 20 mol%)	6.7643374	1.907666	3.55	0.0008*
time	3.2147232	0.976471	3.29	0.0017*
catalyst load* (Time-2.6338)	−2.428134	0.976471	−2.49	0.0156*
equiv **2***catalyst load	4.8499765	2.192979	2.21	0.308*
temp*catalyst load	4.6630511	2.196094	2.21	0.0378*

[a] It is the estimate of the parameter in the model. [b] The t ratio corresponds to the ratio between the parameter estimate and its standard deviation. [c] The effect is statistically significant when the Prob > |t| value is less than 0.05. If this value is more than 0.05 the effect observed is not statistically significant.[16]

Table 3. Sorted parameter estimates across all time points: for the conversion.

Term	Estimate[a]	Std Error	t Ratio[b]	Prob > |t|[c]
con. *rac*-**1a** (0.05, 0.5 M)	11.370995	1.082926	10.50	<.0001*
solvent group (CH_2Cl_2/toluene)	6.4501421	1.030044	6.26	<.0001*
temperature (0, 20 °C)	6.3826351	1.063644	6.00	<.0001*
time	3.0884992	0.530638	5.82	<.0001*
temp*catalyst load	5.184504	0.962113	5.39	<.0001*
equivalents of **2** (0.5, 1 equiv)	5.177096	1.026554	5.04	<.0001*
catalyst load (5, 20 mol%)	4.5743499	1.046247	4.37	<.0001*

[a] It is the estimate of the parameter in the model. [b] The t Ratio corresponds to the ratio between the parameter estimate and its standard deviation. [c] The effect is statistically significant when the Prob > |t| value is less than 0.05. If this value is more than 0.05 the effect observed is not statistically significant.[16]

Table 4. Sorted parameter estimates across all time points: for the enantiomeric excess of the product **3a**.

Term	Estimate[a]	Std Error	t Ratio[b]	Prob > |t|[c]
catalyst group (**IV**, **V**)	15.552599	2.843499	5.47	<.0001*
catalyst load*conc. **1a**	−13.26344	2.803643	−4.73	<.0001*
conc. **1a***catalyst group (**IV**, **V**)	−12.87597	2.843499	−4.53	<.0001*
temp*conc. **1a**	−11.0865	3.064581	−3.62	0.0006*
conc. **1a***(time-2.6)	−4.888808	1.528678	−3.20	0.0022*
temperature (0, 20 °C)	6.8420664	3.064581	2.23	0.0293*
conc. **1a** (0.05, 0.5 M)	5.8890602	2.926153	2.01	0.0487*
time	1.8248673	1.528678	1.19	0.2373*
catalyst load (5, 20 mol%)	2.7841813	2.803643	0.99	0.3247*

[a] It is the estimate of the parameter in the model. [b] The t Ratio corresponds to the ratio between the parameter estimate and its standard deviation. [c] The effect is statistically significant when the Prob > |t| value is less than 0.05. If this value is more than 0.05 the effect observed is not statistically significant.[16]

uct **3a**. The data suggest that the highest enantiomeric excess for compound **3a** can be obtained by employing catalysts **IV** and **V**. Furthermore, it was possible to detect a significant interaction between the solution concentration and the type of catalyst or its loading (see sorted parameters estimates, Table 4).[15] To further optimise simultaneously the system in terms of *ee* of oxazinone **1a** and allyl ester **3a**, another DoE was performed on solvent and catalysts.

Second DoE experiment: Definitive Screening Design (DSD)

The Custom Design allowed us to select two catalysts, two solvents, and to fix the equivalent of allyl alcohol **2** at one equivalent. To obtain optimised reaction conditions for all the responses under study, a second DoE experiment was performed by applying a Definitive Screening Design. This is an efficient screening design that allows evaluation of continuous parameters at three levels (the extreme values of the range under study plus a centre point), giving the possibility of evaluating curvature and generating nonlinear models, whilst keeping the number of experiments low compared with optimization designs that also evaluate parameters at three levels or more but that require many experiments.[17]

The variables investigated were: 1) type of catalyst (categorical variable; two levels: compounds **IV**–**V**); 2) type of solvent (categorical variable; two levels: toluene and dichloromethane); 3) concentration of the solution (continuous variable; three levels: 0.5, 0.25 and 0.05 M); 4) catalyst loading (continuous variable; three levels: 5, 12.5 and 20 mol%), and 5) temperature (continuous variable, three levels: 0, 10 and 20 °C). Time was not included between the variables to study because multiple samples could be taken from the same reaction, which saves time and provides information on the kinetics of the reaction. However, in terms of DoE, the effect of time examined from experiments which are not independent, does not allow this variable to be treated in the same manner as other DoE variables.[18] Thus, 14 randomised experiments were selected by the JMP® programme; the conditions and results are presented in Tables 5 and 6.

Different sets of conditions allowed enantiomeric excess values higher than 90% for oxazinone **1a** and higher than 80% for product **3a** to be obtained (Table 6, entries 1, 4, 11 and 14). With squaramide-derived catalyst **IV** at the highest catalyst loading in dichloromethane, at 0 °C, the reaction was very fast; chiral compound (*S*)-**1a** was obtained with 92% *ee* at 52% of conversion after only 30 min (entry 14). By employing toluene as solvent, lowering the catalyst loading to 12.5 mol%, and running the reaction for 5 h at room temperature, oxazinone (*S*)-**1a** and allyl ester (*R*)-**3a** were obtained with 99 and 82% *ee*, respectively, at 55% conversion with thiourea **V** (entry 4), and 94 and 89% *ee*, respectively, at 51% conversion with 5 mol% of squaramide **IV** (entry 11). Additionally, at 44% conversion, chiral ester (*R*)-**3a** could be obtained with 93% *ee* in the presence of 12.5 mol% **IV**, at 0 °C in a 0.5 M solution after 5 h (Table 5, entry 12).

When a DoE is performed, replication (inclusion of repeated experiments) is used to evaluate experimental variation and re-

Table 5. Reaction conditions for the Definitive Screening Design.

Entry[a]	Cat.	Cat. loading [mol%]	Solution conc. [M]	Solvent	Temp. [°C]
1	**V**	5	0.25	toluene	20
2	**IV**	12.5	0.25	CH_2Cl_2	10
3	**V**	20	0.05	CH_2Cl_2	0
4	**V**	12.5	0.25	toluene	10
5	**V**	20	0.5	toluene	10
6	**IV**	5	0.05	CH_2Cl_2	10
7	**V**	20	0.5	CH_2Cl_2	20
8	**IV**	20	0.05	toluene	20
9	**V**	12.5	0.05	CH_2Cl_2	20
10	**IV**	5	0.05	toluene	0
11	**IV**	5	0.5	toluene	20
12	**IV**	12.5	0.5	toluene	0
13	**V**	5	0.5	CH_2Cl_2	0
14	**IV**	20	0.25	CH_2Cl_2	0

[a] The reactions were performed by employing racemic oxazinone *rac*-**1a** (0.1 mmol) and anhydrous solvents. For the experimental procedure see the Supporting Information.

Table 6. Results of the Definitive Screening Design.

Entry[a]	*ee* [%] after 30 min[b]		Conv. [%][c]	*ee* [%] after 5 h[b]		Conv. [%][c]	S[d]
	1a	**3a**		**1a**	**3a**		
1	31	92	25	94	81	54	32
2	98	81	55	>99	45	>69	12
3	6	99	6	30	93	24	52
4	59	88	40	99	82	55	49
5	87	87	50	99	64	61	22
6	23	94	20	83	90	48	49
7	83	82	50	99	56	64	17
8	5	99	5	21	95	19	22
9	5	99	5	23	93	20	30
10	4	99	4	12	95	11	76
11	48	92	34	94	89	51	70
12	34	94	27	74	93	44	74
13	37	91	29	99.4	75	>57	39
14	92	86	52	99.9	58	63	26

[a] For experimental details see the Supporting Information. [b] Enantiomeric excess was determined by HPLC analysis on a chiral stationary phase. [c] The conversion was determined by HPLC analysis on a chiral stationary phase by comparison with the peak areas of stock solutions of oxazinone *rac*-**1a** in toluene. Quantification was based on UV detection at $\lambda = 230$ nm. [d] Selectivity factor defined by the Kagan equation assuming a first-order reaction and neglecting possible nonlinear effects: S factor $= \ln[(1-C)(1-ee_{\text{starting material}})]/\ln[(1-C)(1+ee_{\text{starting material}})]$.[2f]

producibility of the reaction. Thus, entries 1 and 11 of Table 5 were repeated to include a statistic treatment of the error, validate the models, study reproducibility and, if necessary, correct the models. Each experiment was repeated three times, sampling the reaction after 5 h. The results of each experiment were compared between them to evaluate the reaction reproducibility and with the model predictions to evaluate the reliability of the model itself. Regarding the predictions, the results of the second DoE allowed models to predict the responses of interest. With this data, the JMP® programme was able to generate a prediction profiler that shows how the parameters in the model affect the enantiomeric excess and the conversion. As an example Figure 1 illustrates the predicted responses for the reaction performed under the conditions detailed in Table 5, entry 11. The values of the responses obtained with compound **IV** show that the experiment was reproducible within the experimental error. There was good agreement between experimental and predicted enantiomeric excesses and conversions (*ee* (*S*)-**1a**: predicted 99%; experimental 99, 99 and >99.9%; *ee* (*R*)-**3a**: predicted 84%; experimental 86, 86 and 81%; conversion: predicted 55%, experimental 53, 54 and 55%). For catalyst **V**, the enantiomeric excesses of the oxazinones were slightly higher than the prediction (*ee* (*S*)-**1a**: predicted 94%; experimental 98, 98 and 99%), whereas the enantiomeric excess obtained for the ester were below the prediction (*ee* (*R*)-**3a**: predicted 82%; experimental 74, 77 and 84%).

To correct the models, these data were added in the software to the table of results for the DSD design. New improved models were then generated. The models obtained are only valid within the ranges studied in the DoE, however, they suggested that increasing the temperature and decreasing the amount of catalyst outside of the range might lead to further improvements. Extrapolation of a model may give incorrect predictions if the models do not hold true outside the range. However, we decided to try these new conditions to see whether there was any scope for improvement. Increasing the temperature from 20 to 30 °C whilst simultaneously decreasing

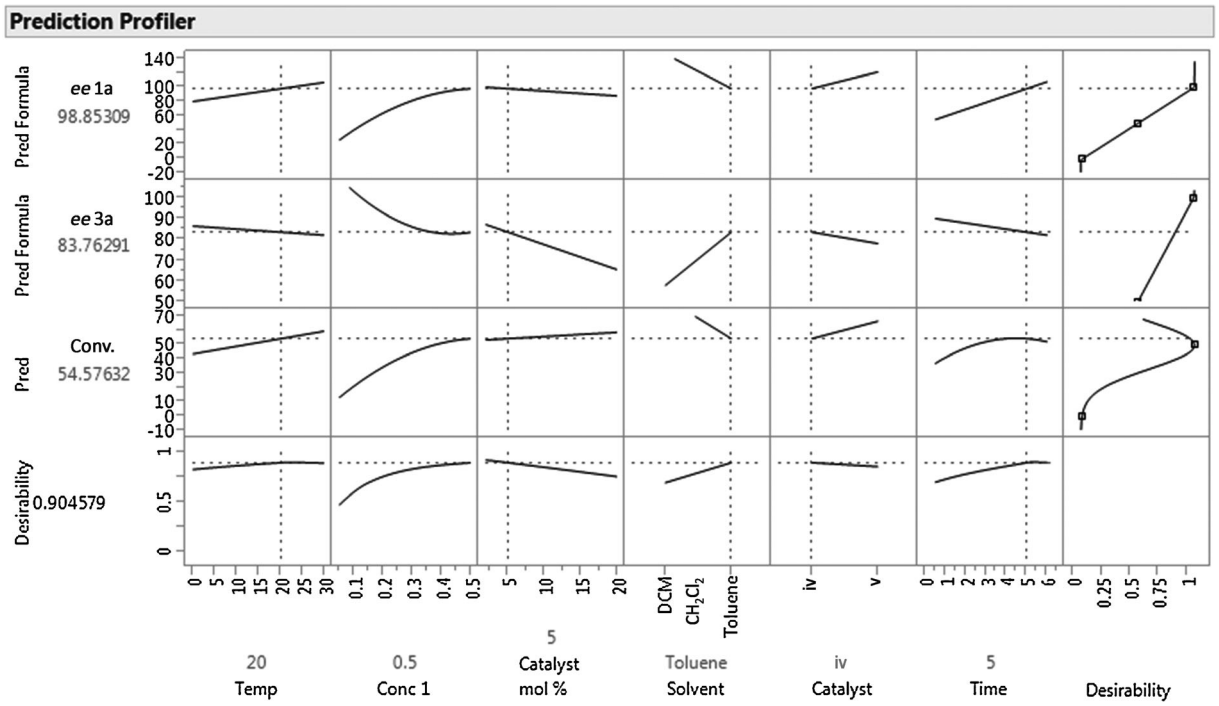
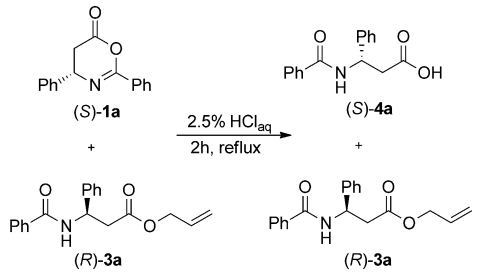


Figure 1. Prediction profiler for the reaction conditions given in Table 5, entry 11.

the catalyst loading to 2 mol% caused a decrease in the enantiomeric excess of the product with both catalysts to 68%. Keeping the temperature at 20 °C, it was possible to decrease the catalyst loading to 3 mol% with a concomitant longer reaction time. Considering the results obtained, we concluded that the models were not able to predict the behaviour of the kinetic resolution outside the ranges studied for the catalyst loading and temperature. With both catalysts, a temperature rise did not lead to an improvement in the enantiomeric excess of the product, but led to lower values with respect to the prediction and control reaction.

With the optimised reaction conditions in hand (5 mol% catalyst **IV**, solution concentration 0.5 M, anhydrous toluene as solvent, room temperature), we decided to test the feasibility of this kinetic resolution outside the glove-box. The results obtained were compared to those of a control reaction run in the glove-box. After 5 h, both reactions were sampled and analysed by HPLC. The results obtained inside and outside the glove-box were reproducible within the experimental error (control reaction: (*S*)-**1a** 99.8% *ee*, (*R*)-**3a** 88% *ee*; reaction outside the glove-box: (*S*)-**1a** 99.3% *ee*, (*R*)-**3a** 89% *ee*), so we decided to scale up the reaction and to extend its scope without the use of a glove-box, thus simplifying the reaction protocol. The β-amino acids produced by this transformation are synthetically useful building blocks. Therefore, it was interesting to perform a reaction on a larger scale to demonstrate its reproducibility and feasibility on a gram scale. We performed a reaction employing oxazinone *rac*-**1a** (1 g) under the optimised reaction conditions. After 5 h, the reaction was sampled for HPLC analysis and quenched with 2.5% aqueous HCl following the work-up procedure developed by Berkessel and co-workers[9] (Scheme 2). At 53% conversion, chiral amino acid (*S*)-**4a**, derived from the ring-opening of oxazinone (*S*)-**1a**, was obtained with 42% isolated yield and 99% *ee*, whereas the chiral allyl ester (*R*)-**3a** was obtained with 49% isolated yield and 87% *ee* (Table 7).



Scheme 2. Acidic work-up.

Table 7. Enantiomeric excesses obtained before and after the work-up protocol.

Entry	Compound	*ee* [%]	Work-up
1	(*S*)-**1a**	99.6	before
2	(*R*)-**3a**	88	before
3	(*S*)-**4a**	99[a]	after
4	(*R*)-**3a**	87	after

[a] The enantiomeric excess of the chiral amino acid (*S*)-**4a** was determined after its conversion into the corresponding allyl ester (*S*)-**3a**.

Table 8. Scope of the reaction with substituted oxazinones *rac*-**1b–g**.

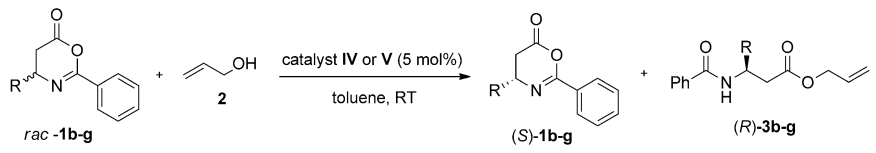
Entry[a]	Cat.	*rac*-**1b–g**	R	Time [h]	*ee* [%][b]		Conv. [%][c]	*S*[d]
					1b–g	**3b–g**		
1	**IV**	**1b**	*p*-MeOC$_6$H$_4$	8	97	90	52	76
2	**IV**	**1c**	*p*-ClC$_6$H$_4$	5	99	80	55	46
3	**IV**	**1d**	*m*-BrC$_6$H$_4$	24	88	69	56	15
4	**V**	**1d**	*m*-BrC$_6$H$_4$	3	98	71	56	34
5	**IV**	**1e**	*i*Pr	24	66	89	43	30
6	**IV**	**1f**	*t*Bu	18	72	81	47	21
7	**IV**	**1g**	*i*Bu	24	88	85	51	35
8	**V**	**1e**	*i*Pr	27	99	83	54	61
9	**V**	**1f**	*t*Bu	48	92	90	51	53

[a] Reaction conditions: *rac*-**1b–g** (0.1 mmol), allyl alcohol **2** (1 equiv) with catalyst **IV** or **V** (5 mol%) in toluene (solution 0.5 M in the presence of catalyst **IV** and 0.4 M with catalyst **V**) at room temperature. [b] Enantiomeric excess was determined by HPLC analysis on a chiral stationary phase. [c] Conversion was determined applying the formula: $C = [ee_{\text{starting material}}/(ee_{\text{starting material}} + ee_{\text{product}})] \times 100$.[2f] [d] Selectivity factor defined by the Kagan equation assuming first-order reaction and neglecting possible nonlinear effects: S factor $= \ln[(1-C)(1-ee_{\text{starting material}})]/\ln[(1-C)(1+ee_{\text{starting material}})]$.[2f]

The dimeric-squaramide **IV** employed as catalyst showed a significant selectivity that was higher than that previously obtained by Berkessel and co-workers employing a thiourea catalyst (Table 9, entry 1). The selectivity factor *S* measured under the best reaction conditions for catalyst **IV** was 98. This improvement is due to the use of a rational approach employing DoE which, using a suitable design, enabled identification of the optimum conditions within the chemical space studied.

Extension of the reaction scope

To explore the scope of our kinetic resolution, the optimal reaction conditions found for the model substrate *rac*-**1a** were applied to oxazinones *rac*-**1b–g** substituted at the 4-position with either aromatic or aliphatic groups (Scheme 3). According to the DoE principles, the optimisation carried out may not be applicable. This means that a DoE screening should have been



R= *p*-OMe-C$_6$H$_4$ *rac*-**1b**, *p*-Cl-C$_6$H$_4$ *rac*-**1c**, *m*-Br-C$_6$H$_4$ *rac*-**1d**, *i*Pr *rac*-**1e**, *t*Bu *rac*-**1f**, *i*Bu *rac*-**1g**

Scheme 3. Scope of the reaction.

performed on each substrate. However, this was outside of the scope of our investigation, therefore we tested the best conditions for the model substrate to evaluate their applicability to *rac*-**1b–g**.

In the presence of catalyst **IV**, the chiral electron-rich *p*-methoxyphenyl and the electron-poor *p*-chlorophenyl substituted oxazinones **1b** and **1c** were obtained with excellent enantiomeric excess, and the corresponding chiral esters **3b** and **3c** were obtained with 90 and 80% *ee*, respectively (Table 8, entries 1 and 2).

The presence of a bromine atom in the *meta*-position of the aromatic ring affected both the enantioselectivity and the reaction rate (Table 8, entries 3 and 4). Sterically more demanding aliphatic *iso*-propyl and *tert*-butyl residues influenced the efficiency of the squaramide catalyst **IV**; after 24 and 18 h, oxazinones (*S*)-**1e–f** could be obtained with only 66 and 72% *ee*, respectively (entries 5 and 6). The enantiomeric excess of the corresponding esters were respectively 89 and 81%. Moderate results were obtained in the kinetic resolution of *iso*-butyl oxazinone *rac*-**1g** in the presence of squaramide-derived catalyst **IV** (entry 7). A short screening showed that better results can be achieved by using catalyst **V** and working with a 0.4 M solution. With the new conditions, oxazinones (*S*)-**1e–f** could be obtained with 99 and 92% *ee*, respectively, after 27 and 48 h. Esters (*R*)-**3e–f** were obtained in 83 and 90% *ee*, respectively (entries 8 and 9).

Table 9 compares the results obtained by following the different approaches. A comparison between entry 1 (results from Berkessel et al.,[9] for which first-generation chiral thioureas were employed) and the best results from Table 1 (for which second-generation *Cinchona* alkaloid-derived bifunctional thioureas were employed) showed that the second-generation chiral thioureas were superior (*S* factor: 45 (entry 2) vs. 35 (entry 1)). The first DoE experiment (reported in the Supporting Information) served as an "information gathering" process. The enantiomeric excess of chiral products **1a–3a** are *apparently* much better with respect to Table 1, but it should be taken into consideration that the selectivity factor *decreases* slightly (*S* factor: 45 (entry 2) vs. 41 (entry 3)). This first DoE (entry 3) served as preliminary campaign to gain insights into the conditions of the reaction. This information was then analysed and exploited to design the second DoE (entry 4), which allowed a remarkable *S* factor of 98 to be achieved, thus facilitating this kinetic resolution (53% conversion, **1a**: 99.6% *ee*; **3a**: 88% *ee*).

Table 9. Comparison of the results for the various approaches towards the kinetic resolution of racemic 2,4-diphenyl-4,5-dihydro-1,3-oxazin-6-one *rac*-**1a** to give allyl ester **3a**.

Entry[a]	Method	*ee* **1a** [%][b]	*ee* **3a** [%][b]	Conv. [%][c]	*S*[d]
1	previous results (ref. [9])	99	86	57	35
2	preliminary catalyst screening results (Table 1)	88	88	50	45
3	first DoE experiment: custom design (see the Supporting Information)	>99	79	56	41
4	definitive screening design (Table 6)	99.6	88	53	98

[a] Reaction conditions: see the Supporting Information and ref. [9] [b] Enantiomeric excess was determined by HPLC analysis on a chiral stationary phase. [c] The conversion was determined by HPLC analysis on a chiral stationary phase by comparison with the peak areas of stock solutions of the oxazinone *rac*-**1a** in toluene. Quantification was based on UV detection at $\lambda = 230$ nm. [d] Selectivity factor defined by the Kagan equation assuming first-order reaction and neglecting possible nonlinear effects: S factor $= \ln[(1-C)(1-ee_{\text{starting material}})]/\ln[(1-C)(1+ee_{\text{starting material}})]$.[2f]

Conclusion

The optimisation of the kinetic resolution of *rac*-**1a** was performed by applying two rational screening designs on four catalysts and five solvents and other variables (catalyst loading, solution concentration, equivalents of nucleophile **2** and temperature). The first screening design allowed the identification of two catalysts and two possible solvents as the most promising conditions to yield simultaneously both the starting material **1a** and the allyl ester product **3a** with the highest enantiomeric excess. The second screening (DSD design) allowed the optimised reaction conditions to be established, reaching 99.6% *ee* for oxazinone (*S*)-**1a** and 88% *ee* for the product (*R*)-**3a** at 53% conversion (catalyst **IV** selectivity factor $S = 98$). The reaction was performed on 1 gram scale of starting-material without the need for a glove-box. We also confirmed that the established conditions could be a good starting point for the kinetic resolution of a number of substituted oxazinones *rac*-**1b–g**.

It was shown, using a previously reported kinetic resolution as a model reaction, that a rational approach such as DoE, can be a powerful tool with which to optimise asymmetric reactions. Statistic treatment should also be encouraged for adoption by organic chemists in academia. Carlson and Carlson stated that "statistics is always secondary to chemistry in the domain of organic synthesis. It does not matter how statistically significant an analysis turns out to be if the chemistry does not afford the desired results. Therefore, any conclusion from a model must be confirmed by an experiment."[19]

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Keywords: design of experiments · kinetic resolution · organocatalysis · squaramides · thioureas

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[10] The control reaction was performed by employing *rac*-**1a** (0.1 mmol) and allyl alcohol **2** (1 equiv) in anhydrous toluene (0.85 mL). The reaction was sampled for HPLC analysis. The HPLC trace showed the formation of 0.7% of product *rac*-**3a** after 5 h.

[11] The scope is the application of DoE, hence a limited and nonexhaustive set of catalysts was chosen for the purpose of this investigation. For the synthesis of the catalysts employed, see: a) G. Kardos, T. Soós, *Eur. J. Org. Chem.* **2013**, 4490 – 4494 (catalyst **I**); b) W. Yang, D.-M. Du, *Org. Lett.* **2010**, *12*, 5450 – 5453 (catalyst **II**); c) Y. Qian, G. Ma, A. Lv, H.-L. Zhu, J. Zhao, V. M. Rawal, *Chem. Commun.* **2010**, 3004 – 3006 (catalyst **III**); d) J. Woong Lee, T. Hi Ryu, J. Suk Oh, H. Yong Bae, H. Bin Jang, C. Eui Song, *Chem. Commun.* **2009**, 7224 – 7226 (catalyst **IV**); e) see also ref. [7] (catalysts **V** – **VI**); f) B. Vakalya, S. Varga, A. Csámpau, T. Soós, *Org. Lett.* **2005**, *7*, 1967 – 1969.

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[13] The synthesis of substrates *rac*-**1a**–**g** was improved with respect to the previously reported procedure (see the Supporting Information, ref. [8]) replacing *iso*-butyl chloroformate with thionyl chloride to promote the ring-closure of the protected amino acids *rac*-**5a**–**g**. The use of thionyl chloride was advantageous because the products could be purified without the need for column chromatography on silica gel. For detailed information, see the Supporting Information.

[14] JMP 11®; statistical software package is available from SAS.

[15] For the Custom Design table of experiment, results and full models, see the Supporting Information.

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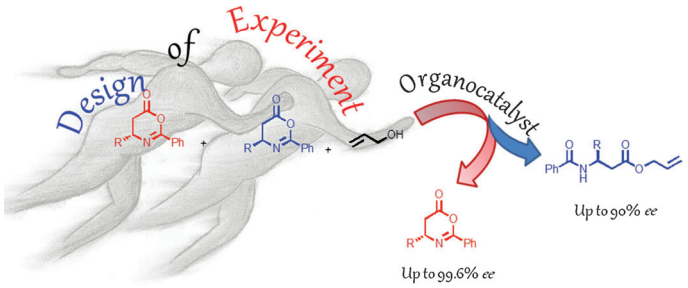

Rational optimization: Kinetic resolution of oxazinones has been studied as a model reaction to develop a sound strategy to achieve the best results with a rational approach rather than by trial-and-error (see scheme). With this strategy, chiral oxazinones can be resolved with *ee* values up to 99.6% and ester products with *ee* values up to 90% (selectivity factor up to $S = 98$).

Kinetic Resolution

*P. Renzi, C. Kronig, A. Carlone, S. Eröksüz, A. Berkessel, M. Bella**

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Kinetic Resolution of Oxazinones: Rational Exploration of Chemical Space through the Design of Experiments