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Automated Process Research and the Optimization of the Synthesis of 4(5)-(3-Pyridyl)imidazole

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Abstract:

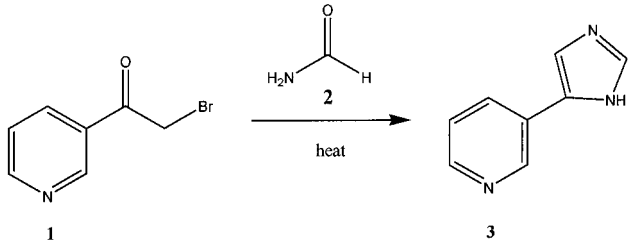
Automated process development technology was applied to the synthesis of 4(5)-(3-pyridyl)imidazole. This method utilizes automated liquid handling equipment coupled with statistically designed protocols for rapid process optimization. Two experimental sets were carried out based on a three-level factorial and central composite designs to optimise the product yield. The central composite design was repeated on one-fifth the scale to test the capabilities of the automated equipment. The reaction variables investigated were temperature and stoichiometry of formamide. The optimum in situ yield of 4(5)-(3-pyridyl)-imidazole was found to be at 160 °C and 9 equiv of formamide. The results from the automated technology can be applied to larger-scale synthesis of the desired compound.

Introduction

Imidazole rings are found in numerous natural compounds such as enzymes, nucleic acids, and alkaloids that play a role in biological processes.[1] Imidazole-containing compounds are synthetic targets for the pharmaceutical industry. For example, compounds derived from histamine and possessing an aromatic nitrogen-containing heterocycle on the side chain amino group show H_3-antagonist activity.[2] There are many articles on the synthesis of various substituted imidazole groups.[1,3−5] C-substituted imidazoles can be prepared from diketones, α-halogenoketones, α-hydroxyketones, α-aminoketones, carbohydrates, and oxazoles with reagents such as ammonia, formamide, and formamidine.[1]

The condensation/cyclization of α-diketones, α-hydroxyketones, α-halogenoketones, α-aminoketones, α-haloesters, or α-oximinoketones with excess formamide at high temperatures is known as the Bredereck reaction (Scheme 1).[4−7] This route has been successful in the preparation of 4,5-disubstituted imidazoles with yields varying between 40 and 90%.[4−7] However, the reproducibility of this method has been debated, and some investigators found yields as low as 20−30% for various 4,5-disubstituted imidazoles.[4,5]

Scheme 1



Traditional optimization of reaction conditions is often a time-consuming process requiring examination of one variable at a time. The optimum reaction conditions can be much more rapidly determined using automated process research (APR) technology.[8−12] APR combines automated parallel experimentation equipment with statistical experimental design to provide libraries of reaction conditions. Researchers can rapidly examine multiple variables in a defined series of reactions. This minimizes the number of experiments that are necessary for optimization on a large scale. As a result, the development time and costs are significantly reduced.[8−12] There are several applications of automated process research. The most common are chemical scale-up, chromatography/purification, materials science, and personal care/consumer products.[8]

Our strategy was to perform a statistically designed optimization of the synthesis of 4(5)-(3-pyridyl)imidazole (**3**) using the automated research technology described above. We initially developed a synthetic route for the formation of **3** involving the Bredereck reaction with 2-bromo-1-(3-pyridyl)-1-ethanone (**1**) and formamide (**2**) to afford the desired product **3** in only 20% yield.[13] We were interested in applying rapid process optimization technology to improve the overall yield of **3**. Previous bench experiments suggested that the experimental variables that require optimization were reaction temperature and the stoichiometry of **2**.[13] The concentration of **1** and reaction time were held constant, and the reaction temperature and equivalents of formamide were

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varied over a statistically selected range. Our results can be applied for the scale-up of the desired compound. The accuracy of the liquid transfer and weighing equipment was also tested with various solvents.

Experimental Procedures

Materials. All chemicals and HPLC solvents were of the highest quality that was commercially available and used without further purification. Compound **1** was a generous gift from Condeiu and co-workers.[13]

General Procedures. Three sets of experiments were carried out to optimize the synthesis of **3** and to test the limitations of the automated equipment. The experimental designs were generated using Design Expert software (Stat-Ease, Inc.) Liquid transfer and weighing operations were carried out with a Gilson 215 liquid handler and a Bohdan RAM automated workstation, respectively. The reaction temperatures were regulated with a Watlow thermal control unit. The reaction time for all experiments was 7 h. Samples were dried with a Savant SpeedVac. In situ yields were quantified by HPLC on a Gilson Combinatorial Chromatography System (column: 250 × 46 mm Chiralpak AD). A sample of **3** (greater than 99% by LC/MS) that was synthesized by Condeiu and co-workers[13] was used as an authentic standard for HPLC studies. The run conditions were as follows: isocratic HPLC method with the solvent containing 84% acetonitrile, 15% water, and 1% trifluoroacetic acid; flow rate of 1 mL/min; 254 nm UV detection. The data were analyzed using Design Expert software.

Liquid Transfer Procedure. Three solvents were investigated to test the lower limits on liquid transfer and weighing capabilities for the Gilson 215 liquid handler and the Bohdan workstation, respectively. Ten 8 mL vials with septa caps were weighed utilizing the Bohdan workstation. To these vials, 10 to 7000 μL of solvent was added by the Gilson 215 liquid handler or the Bohdan workstation. This procedure was repeated in triplicate. The net weight for the solvent in each vial was determined using the Bohdan workstation. The average weight was calculated and corrected for density. The weights were then compared to the theoretical amounts of liquid that were dispensed. The Gilson 215 liquid handler was tested utilizing two syringe and liquid loop sizes (1 mL syringe, 1.5 mL liquid loop, and 10 mL syringe, 10.5 mL liquid loop). The dispensing rate for both instruments was held constant at 2 mL/min.

Experimental Designs. A three-level factorial design was the basis for the first experimental plan that was proposed using Design Expert software. Thirteen experiments, consisting of eight reactions with combinations of different temperatures and equivalents of formamide as well as five center points (T = 140 °C; 5 equiv of **2**) were carried out. All reaction mixtures contained 500 mg of **1**. The temperature range was 110−170 °C and the stoichiometric ratio for **2** was varied from 2 to 8 equiv. The reactions were conducted in three blocks according to their respective temperatures (Table 1).

A central composite design was implemented to further optimize the synthesis of **3** in the second experimental plan.

Table 1. **Synthesis of 4(5)-(3-pyridyl)imidazole based on the three-level factorial design (experimental plan 1)**

experiment number	block number	temperature (°C)	equiv of **2**	% yield of **3** (in situ)
7	1	110	2	5
11	2	140	2	11
8	3	170	2	9
10	1	110	5	33
2	2	140	5	57
4	2	140	5	54
6	2	140	5	59
9	2	140	5	58
12	2	140	5	59
3	3	170	5	58
5	1	110	8	42
13	2	140	8	69
1	3	170	8	67

Table 2. **Synthesis of 4(5)-(3-pyridyl)imidazole based on the central composite design (experimental plan 2)**

experiment number	block number	temperature (°C)	equiv of **2**	% yield of **3** (in situ)
6	1	132	8.0	61
8	2	140	6.0	54
9	2	140	10.0	66
11	3	160	5.2	52
1	3	160	8.0	73
4	3	160	8.0	76
5	3	160	8.0	71
12	3	160	8.0	72
13	3	160	8.0	74
10	3	160	10.8	67
3	4	180	6.0	48
2	4	180	10.0	59
7	5	188	8.0	64

A set of 13 individual experiments contained eight reactions with combinations of different reaction temperatures and equivalents of **2** as well as five center points (T = 160 °C; 8 equiv of **2**). This experimental plan was based on the statistical model analysis of the three-level factorial design using Design Expert software. Reaction mixtures contained 500 mg of **1**. The temperature range and stoichiometric ratio of **2** were 132−188 °C and 5.2−10.8 equiv, respectively. The reactions were carried out in five blocks according to their respective temperatures (Table 2). A similar design was utilized for the third set of experiments, but the reaction scale was reduced by a factor of 5 to test the lower limits on liquid transfer and weighing.

Synthesis of 4(5)−(3-Pyridyl)imidazole under Automated Conditions. The reactions were carried out in 18 mL screw-top tubes equipped with permeable caps. The appropriate amount of **2** was transferred to the vials containing **1** to initiate the reaction. After heating for 7 h, the reactions were cooled to room temperature. Methanol was added to transfer the samples to preweighed vials. The samples were dried under vacuum, and the yield was estimated by weighing the vials following solvent removal. Approximately 50 mg of each product **3** was used for HPLC analysis to quantify yield and determine the level of purity. The data were then analyzed to determine the optimum reaction conditions.

Results and Discussion

Investigators have reported yields of 4,5-disubstituted imidazole compounds ranging from 20 to 90% for the Bredereck reaction. Using traditional bench chemistry, the small-scale reaction of **1** with 2 equiv of **2** at 150 °C afforded **3** in 20% yield.[13] This low yield is more consistent with the results of Grimmett and co-workers, whereas other researchers reported yields from 40 to 90% for related 4,5-disubstituted imidazoles.[4,5]

Three sets of experiments were performed using automated process technology to optimize the reaction conditions for the synthesis of **3**. A three-level factorial design was the basis of the first set of experiments generated by Design Expert software (Stat-Ease, Inc.). The reaction temperature and the stoichiometry of **2** were varied to optimize the in situ yield of **3**. The amount of **3** was quantified by HPLC analysis using a highly pure sample of **3** as the authentic standard. The reaction conditions and in situ yield of **3** are listed in Table 1.

The data from Table 1 were best described by a quadratic equation using Design Expert software (Yield = (3.31 × Temp) + (18.25 × Eq) − (0.012 × Temp^2) − (1.79 × Eq^2) + (0.058 × Temp × Eq) − 262.7). This model had a $P > F$ of less than 0.0001 and a correlation coefficient (R^2) equal to 0.985. The data corresponding to the five center points (experiment numbers 2, 4, 6, 9, and 12) demonstrated an rsd of 2% at a confidence level of 90%. The outlier point corresponding to reaction 7 (5% of **3**) was not excluded from the model. This reaction was in the lowest-temperature region of the design space and does not significantly affect the data analysis. The similarity between the five center points indicates that the automated reactions are reproducible. The three-dimensional (3-D) contour plot of the three-level factorial design is illustrated in Figure 1. The *X* and *Y* variables represent the reaction temperature (Temp) and equivalents of formamide (Eq.), respectively, and *Z* represents the statistically predicted yield of **3** based on the quadratic model. This model indicates that the yield of **3** increases as the reaction temperature is increased from 110 to 155 °C and decreases at higher temperatures. As the equivalents of **2** are increased from 2 to 8, the yield increases rapidly. The optimum reaction conditions are located on the very limits of the design space at 155 °C and 8 equiv of **2**.

On the basis of these results, a central composite design was implemented in the second experimental plan to further optimize the reaction conditions. The reaction temperature and the stoichiometry of **2** were varied from 132 to 188 °C and 5.2−10.8 equiv, respectively. The reaction conditions and the in situ yield of **3** are outlined in Table 2. Utilizing the Design Expert software, the data were once again fitted to a quadratic equation (Yield = (5.13 × Temp) + (35.41 × Eq) − (0.016 × Temp^2) − (1.98 × Eq^2) − (0.00625 × Temp × Eq) − 485.15). The $P > F$ and R^2 values are 0.0015 and 0.91, respectively. The outlier point corresponding to reaction 7 (64% of **3**) was not excluded from the model, as it did not significantly affect the data analysis. An overlay for the central composite design is presented in Figure 2. The percent yield of **3** increases as temperature is increased from 132 to 160 °C and the equivalents of **2** are increased from 5.2 to 9. At higher temperatures and equivalents of **2**, there is a decrease in the amount of **3**. The yield of **3** is optimal at a temperature of 160 °C and 9 equiv of formamide. The reaction optimum is within the design space for the second experimental plan, with a predicted maximum in situ product yield of 74 ± 3%.

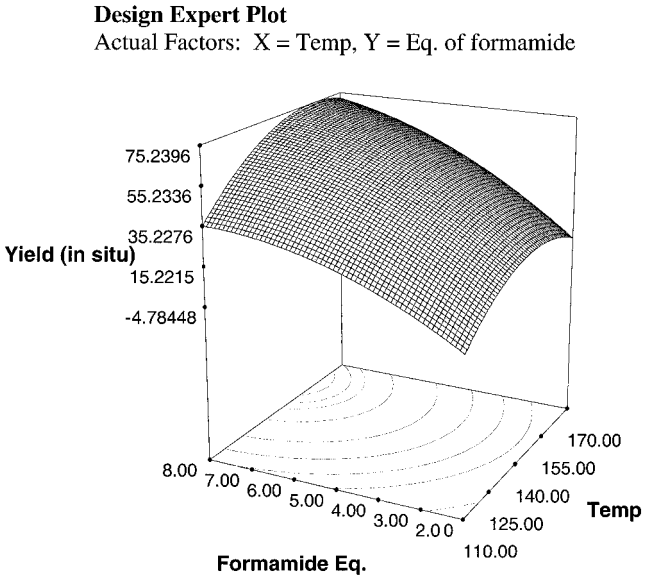

Figure 1. **The contour plot of experimental plan 1 that was based on a three-level factorial design. The *X*, *Y*, and *Z*-variables are the reaction temperature (Temp), equiv (Eq.) of formamide, and the statistical in situ yield of 3, respectively. The reaction optimum conditions with a temperature of 155 °C and 8 equiv of formamide are located on the very limits of the design space.**

The central composite design was repeated on one-fifth the scale to test the liquid-dispensing capabilities of the automated equipment. Only 50−150 μL of **2** were dispensed for the small-scale reaction. The optimum reaction conditions for the third experimental plan were 160 °C and 8 equiv of **2** (data not shown). Although these conditions are similar to those for the second experimental plan (500 mg of **1**), the in situ yields were approximately 15% lower. This error is most likely due to lower limits in the dispensing capabilities of the automated equipment.

The accuracies of liquid transfer for the Gilson 215 handler and the Bohdan workstation were previously tested by the addition of the solvents water, THF, and dichloromethane to preweighed 8 mL vials. The amount of dispensed solvent ranged from 10 to 7000 μL. The percent error for the dispensing volume was constant from 100 to 7000 μL for all three solvents. When the dispensing volume of the Gilson 215 handler was equal to 50 μL, the percent error for THF and dichloromethane was 30 and 18%, respectively. Not surprisingly, the least volatile solvent, water, was dispensed with the highest degree of accuracy, and significant error was not observed until the dispensing volume was below 50 μL. The results indicated that at dispensing volumes of less than 100 μL, there was a steep decline in the accuracy of the liquid-transfer equipment for

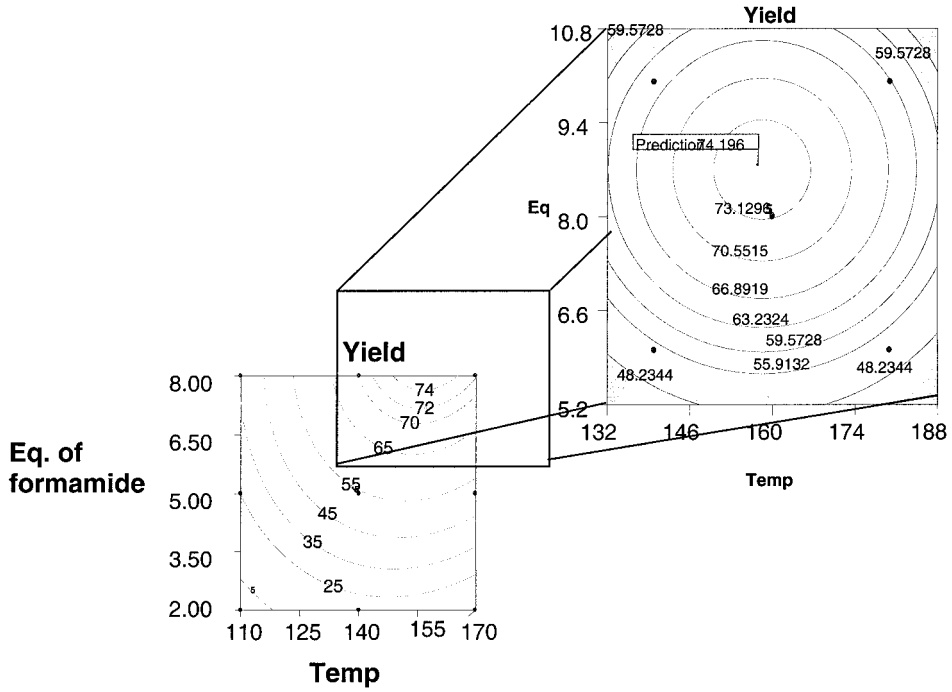

Figure 2. **Overlay plot for the central composite design. The *x* and *y* axis correspond to the reaction temperature and the equiv (Eq.) of formamide. The statistical yields are shown within the plot. On the basis of the quadratic model, a 74% yield is predicted. The reaction optimum conditions are displayed within the central composite design space with a temperature of 160 °C and 9 equiv of formamide.**

volatile solvents. Similar errors were observed for the weighing equipment that may have affected the in situ yields for the third experimental plan.

The coupling of statistical design and automated equipment can be used as a tool for rapid optimization of reaction conditions. An initial small-scale Bredereck reaction of **1** and **2** afforded 20% yield of **3** prior to rapid process optimization.[13] The critical variables for yield optimization were temperature and the number of equivalents of **2**.[13,14] We have demonstrated the use of APR technology for the optimization of the reaction conditions. This research greatly reduces the amount of time associated with traditional optimization techniques. The optimum in situ yield of **3** (74%) was found to be at 160 °C and 9 equiv of formamide. There is a drastic change in the yield when either the equivalents of **2** or the temperature are varied. For example, when $T = 160$ °C with 5.2 equiv of **2**, the yield of **3** decreases by greater than 20%. If the temperature is varied at 8 equiv of **2**, there is an approximate 10% decrease in **3**. The results from rapid process optimization can be applied to the chemical scale-up of the reaction.

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Conclusions

The main focus of our study was to blend existing combinatorial techniques, automated equipment, and statistical process design into a rapid protocol for process optimization and variable screening. Overall, the automated protocol functioned well, providing an initial information base of desired reaction conditions. The results from the designed experimental plans can be implemented into a scale-up production of **3**.

Our future plans are to refine this procedure to increase the links between the automated synthesis equipment, analysis instrumentation, and the statistical design software. The automated equipment has the ability to handle hundreds of reactions. This platform is well-suited for rapid chemical process screening experiments.

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