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Automated Process Research. An Example of Accelerated Optimization of the Friedel–Crafts Acylation Reaction, a Key Step for the Synthesis of Anti-HIV (+)-Calanolide A

Jintao Zhang,* Eric W. Kirchhoff, David E. Zembower, Nancy Jimenez, Prabir Sen, Ze-Qi Xu, and Michael T. Flavin
MediChem Research Inc., 12305 South New Avenue, Lemont, Illinois 60439, U.S.A.

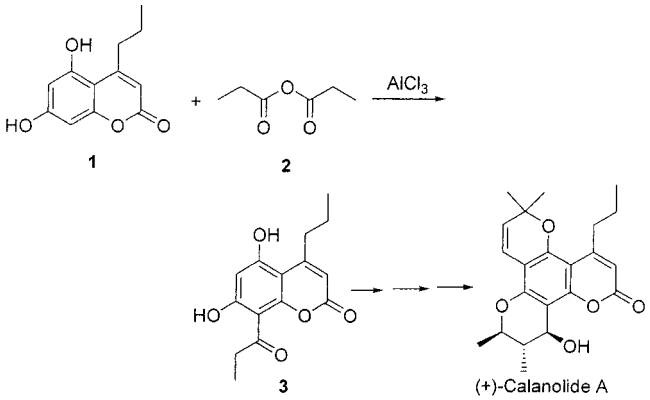
Abstract:

An automated process research approach to reaction optimization was developed. Chemical process research can be greatly accelerated by coupling automated synthesis equipment with statistical design of experiments (DoE). With the use of an automated process approach, multiple experiments can be performed in parallel on an automated platform, and multiple parameters that may influence process performance can be examined within one set of experiments generated from statistical design. We have successfully applied an automated process research approach to optimize the Friedel–Crafts acylation reaction that was used in our total synthesis of (+)-calanolide A, a potential anti-HIV agent currently in clinical trials. The in situ yield for a coumarin product was successfully optimized, increasing from 70% to 97% by HPLC analysis.

Introduction

(+)-Calanolide A,[1] isolated from the dried fruits and twigs of *Calophyllum lanigerum* var. *Austrocoriaceum*, is a non-nucleoside reverse transcriptase inhibitor currently in clinical trials as an anti-HIV agent. Our synthetic route for the large-scale preparation of (+)-calanolide A involves a five-step synthetic sequence.[2] A Friedel–Crafts acylation reaction, illustrated in Scheme 1, is used to generate 5,7-dihydroxy-4-propyl-8-propionylcoumarin (**3**) as a key intermediate for the synthesis of (+)-calanolide A. This reaction has typically been conducted by treating 5,7-dihydroxycoumarin (**1**) with 1 equiv of propionic anhydride in the presence of 3 equiv of $AlCl_3$ in refluxing dichloroethane (DCE). The in situ yield for coumarin **3** averaged 70% by HPLC analysis, and the typical isolated yield was 45%. Small amounts of the side products, i.e., 5,7-dihydroxy-4-propyl-6-propionylcoumarin and 5,7-dihydroxy-4-propyl-6,8-dipropionylcoumarin, as well as the starting material were found in the reaction mixture.

Scheme 1



The experimental conditions used in the preparation of coumarin **3** may not be optimal in terms of yield, purity, or cost-effectiveness. When using the traditional approach to reaction optimization, which involves a stepwise examination of reaction parameters, a large number of experiments may be necessary to adequately investigate the wide range of parameters that may influence process performance. This approach can be a very time-consuming and repetitive task. With the help of automated synthesis equipment coupled with statistical design of experiments (DoE), the reaction optimization process can be dramatically accelerated by performing multiple experiments in parallel on an automated platform. The effects of multiple parameters on process performance can be investigated from one set of statistically designed experiments. Indeed, during the course of our study, other reports describing the automated process research approach were published.[3] For example, Wagner et al.[3a] reported automated screening of cocatalysis conditions using an automated microscale multireactor workstation; Emiabata-Smith et al.[3b] described the development, operation, and application of an automated workstation for performing solution-phase organic synthesis and online HPLC analysis.

This paper describes our development of a rapid automated process research approach to reaction optimization coupled with the application of statistical DoE. The effects of different Lewis acids, solvents, reaction temperatures, and stoichiometry of Lewis acids on the yield of coumarin **3** for the Friedel–Crafts acylation reaction were investigated.

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Results and Discussion

Our first goal was to demonstrate the reliability and reproducibility of the reaction conducted under standard conditions, but using automated synthesis equipment. The reaction was conducted on a 250-mg scale (coumarin **1**) with 1 equiv of propionic anhydride in the presence of 3 equiv of $AlCl_3$ in 2 mL of dichloroethane (DCE) at 65 °C. One set of four experiments was worked up by the conventional workup procedure; i.e., the reaction mixture was quenched by ice water and extracted with ethyl acetate. Another set of six experiments was worked up using the automated process research procedure described in the Experimental Section. The in situ yield of coumarin **3** was determined by HPLC analysis, which demonstrated that the yield of **3** reached a maximum after 2 h. The average yield of **3** was 65% for the reactions worked up using the conventional procedure ($n = 4$, relative error ±4.9%, relative standard deviation 4.6%) and 63% for the reactions worked up using the automated process research procedure ($n = 6$, relative error ±5.9%, relative standard deviation 4.9%). These data confirmed that the results from the reactions conducted on a small-scale parallel format were consistent with the results obtained on traditional bench scale[2b] (70% in situ yield by HPLC). In addition, the results from the automated process research method were consistent with those from the conventional method. The data from the reactions conducted on a small-scale parallel format were reproducible.

Our initial optimization approach was to examine the effects of various Lewis acids on reaction yield and purity. A set of 24 Lewis acids was randomly selected to screen as potential candidates for the reaction (LaF_3, $TiCl_2$, $TiCl_4$, $TiBr_4$, VCl_3, $NbCl_5$, $NbBr_5$, $TaCl_5$, $MoCl_5$, WCl_6, $FeCl_2$, $FeCl_3$, $ZnCl_2$, BCl_3, BBr_3, $AlCl_3$, $AlBr_3$, $GaCl_2$, $GaCl_3$, $GaBr_3$, $InCl_3$, $SnCl_4$, SbF_5, and $SbCl_5$). The reactions were conducted with 1 equiv of propionic anhydride in the presence of 3 equiv of Lewis acid in DCE at 65 °C. The results indicated that the reaction using either $AlCl_3$ or $AlBr_3$ resulted in the highest in situ yield of coumarin **3**, i.e., 61% for the reaction with $AlCl_3$ and 68% for the reaction with $AlBr_3$, respectively. The remaining 22 Lewis acid-catalyzed acylation reactions gave in situ yields ranging from 0 to 45%. Due to cost considerations and our prior experience, $AlCl_3$ was selected as the optimal Lewis acid for further reaction optimization.

Design Expert software (Stat-Ease, Inc.) was utilized to generate a series of experiments according to a statistical design in order to investigate the effects of solvent, reaction temperature, and $AlCl_3$ stoichiometry on product formation. In addition to DCE, nitromethane was investigated as the reaction solvent, based upon prior literature reports indicating that nitroalkanes are superior solvents for Friedel−Crafts acylations.[4]

A set of 13 experiments was proposed for the reaction in nitromethane with a two-factor, three-level factorial design. The two variables were reaction temperature (ranging from 40 to 90 °C) and stoichiometry of $AlCl_3$ (ranging from 1.0 to 10 equiv). The 13 designed experiments contained eight reactions with combinations of different reaction temperatures and number of equivalents of $AlCl_3$, as well as five center points, i.e., the data point with 5.5 equiv of $AlCl_3$ at 65 °C. The conditions for the 13 experiments proposed by the Design Expert software, as well as the in situ yields for coumarin **3**, are presented in Table 1 for the nitromethane trials. Of particular note, the five center points (entries 5, 7, 8, 9, and 10) indicated excellent reproducibility of the experimental data.

Table 1. **Dependence of the yield of coumarin 3 (%) on reaction temperature (*T*, °C) and number of equivalents of $AlCl_3$ (equiv) for the reaction in nitromethane**

entry	*T* (°C)	$AlCl_3$ (equiv)	yield of **3** (%)
1	40	1.0	2.7
2	40	5.5	63
3	40	10	52
4	65	1.0	2.6
5	65	5.5	73
6	65	10	69
7	65	5.5	71
8	65	5.5	68
9	65	5.5	72
10	65	5.5	72
11	90	1.0	6.9
12	90	5.5	84
13	90	10	82

[a] 250-mg scale reaction in 2 mL of nitromethane.

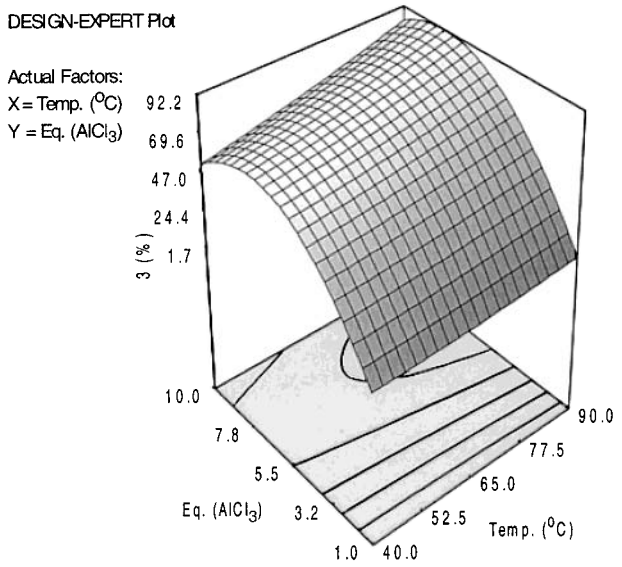

Figure 1. **3-D surface plot from the quadratic model, showing the effects of temperature and number of equivalents of $AlCl_3$ on the in situ yield of coumarin 3.**

The data in Table 1 were analyzed with the use of the Design Expert software, which identified a quadratic model to be the best fit for the data. A three-dimensional (3-D) surface plot, illustrated in Figure 1, was generated according to the quadratic model. The *X*- and *Y*-axes in Figure 1 represent reaction temperature and number of equivalents, respectively, and the *Z*-axis represents the yield of coumarin **3** predicted by the quadratic model. The 3-D surface plot in Figure 1 illustrates that the yield of coumarin **3** increases slightly as the reaction temperature is increased from 40 to

(4) *Friedel−Crafts and Related Reactions*; Olah, G. A., Ed.; Wiley & Sons: New York, 1973; p 313.

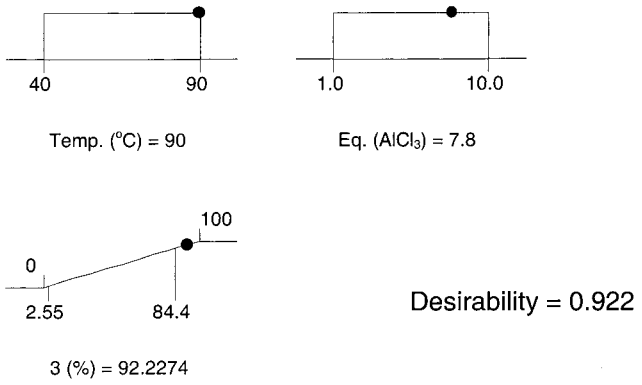

Figure 2. **Optimal reaction conditions for the acylation reaction in nitromethane predicted by the plot in Figure 1.**

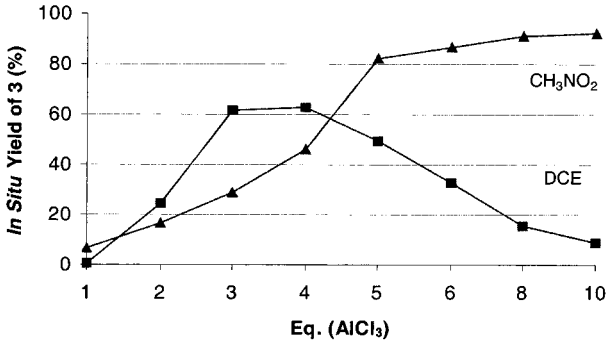

Figure 3. **Dependence of the in situ yield of coumarin 3 (%) on the number of equivalents of $AlCl_3$ for reaction in DCE (■) at 65 °C and in nitromethane (▲) at 90 °C.**

90 °C; the yield of coumarin **3** increases rapidly as the number of equivalents of $AlCl_3$ increases from 1.0 to 8.0 equiv. Further increases in the number of equivalents of $AlCl_3$ led to a slight decrease in the yield of coumarin **3**. The 3-D surface was used to predict optimal reaction conditions for the Friedel−Crafts acylation reaction in nitromethane, as illustrated in Figure 2. The optimal yield for coumarin **3** is predicted to be 92% if the reaction is conducted with 7.8 equiv of $AlCl_3$ at 90 °C in nitromethane.

A similar set of experiments was proposed with the use of the Design Expert software to investigate the effects of reaction temperature (varying from 35 to 65 °C) and number of equivalents of $AlCl_3$ (varying from 1.0 to 10 equiv) on the yield of **3** for the reaction in DCE. A similar 3-D surface plot was obtained for the DCE trials. However, the optimal yield for coumarin **3** was predicted to be only 46% if the reaction is conducted with 6.4 equiv of $AlCl_3$ at 65 °C in DCE. Clearly, changing solvents to nitromethane represented a significant improvement in reaction performance.

A third set of experiments was proposed to examine the optimal conditions predicted by the 3-D surface plots for the reaction in DCE and nitromethane. Figure 3 illustrates the dependence of the yield of coumarin **3** on the number of equivalents of $AlCl_3$ for the reactions at 65 °C in DCE and at 90 °C in nitromethane, respectively. The plot in Figure 3 indicates that the highest yield for coumarin **3** was 65% when the reaction was conducted with 3.5 equiv of $AlCl_3$ in DCE. This result was consistent with the previous result obtained

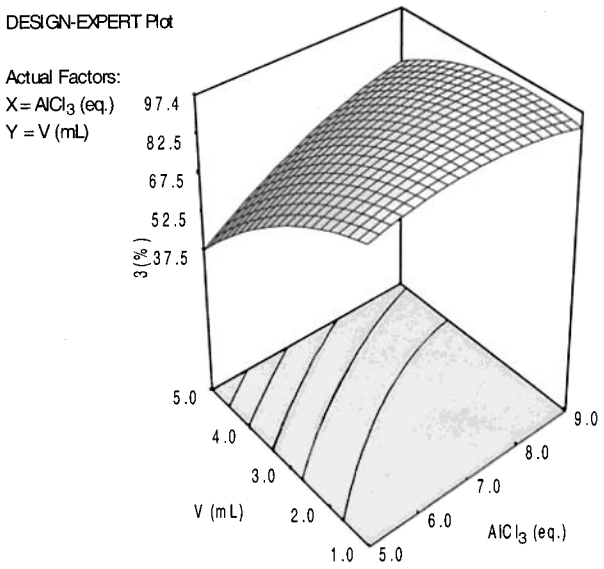

Figure 4. **3-D surface plot from the quadratic model, showing the effects of volume of nitromethane and number of equivalents of $AlCl_3$ on the in situ yield of coumarin 3.**

from the reaction optimization conducted on traditional bench scale. The difference between the predicted data from the model (6.4 equiv of $AlCl_3$, in situ yield 46%) and the experimental data (3.5 equiv of $AlCl_3$, in situ yield 65%) in DCE indicated that the resolution of the model in DCE might not be great enough for generation of accurate predictions. However, the model in DCE provided hints and directions that may lead to optimal reaction conditions.

The plot in Figure 3 also indicates that the yield of coumarin **3** increases as the number of equivalents of $AlCl_3$ increases when nitromethane is used as the solvent. The in situ yield of coumarin **3** was 90% when the reaction was conducted with 7 equiv of $AlCl_3$ in nitromethane. This result was consistent with the predictions from the model. The increase in the stoichiometry of $AlCl_3$ for the reaction in nitromethane indicated that nitromethane may have formed addition complexes with $AlCl_3$.[4] It has been reported[4] that the complex formation between nitromethane and $AlCl_3$ decreases the tarring and disproportionation action frequently caused by $AlCl_3$, thereby making the introduction of acyl groups essentially a mild reaction resulting in greatly improved reaction conversions in nitromethane, but not in DCE. Since part of $AlCl_3$ was consumed by solvent (nitromethane) when the acylation reaction was conducted in nitromethane, the amount of nitromethane may also be one of the important factors that affect the acylation reaction. Therefore, a final set of experiments was designed to investigate the effects of the amount of nitromethane and the number of equivalents of $AlCl_3$, i.e., $AlCl_3$ concentration, on the yield of coumarin **3** prepared in nitromethane.

The final set of reactions was conducted in nitromethane at 90 °C using 250 mg of **1** with varying quantities of $AlCl_3$ (5.0−9.0 equiv) in varying volumes of solvent (1.0−5.0 mL), and the resulting 3-D surface plot is illustrated in Figure 4. The plot in Figure 4 illustrates that the yield of coumarin **3** increases as the volume of nitromethane decreases from 5.0 to 1.0 mL. The yield of coumarin **3** varies between 92%

and 97% as the number of equivalents of $AlCl_3$ is increased from 5.0 to 9.0 equiv when the reaction was carried out in 1.0 mL of nitromethane. The optimal reaction condition predicted by the model in Figure 4 was the use of 7 equiv of $AlCl_3$ in 1.0 mL of nitromethane, with a predicted yield of 97%. However, considering the fact that the yield of coumarin **3** does not change much (between 92% and 97%) when the number of equivalents of $AlCl_3$ is decreased from 9 to 5 equiv, we would expect that the use of 5 equiv of $AlCl_3$ in 1 mL of nitromethane should also give an excellent yield of coumarin **3**.

By combining the conclusions of the individual studies above, the predicted optimal reaction condition involved the use of 5 equiv of $AlCl_3$ in 1.0 mL of nitromethane (250-mg scale) at 90 °C. To confirm this prediction, the acylation reaction was conducted on 2.5- and 10-g scales. For the reaction on a 2.5-g scale, the isolated yield of crude product was 86%, and the purity of the crude product was 95% by HPLC analysis. For the reaction on a 10-g scale, the in situ yield for coumarin **3** was 97% by HPLC analysis, and the yield of the crude product was 91%. These results are dramatically superior to those experienced prior to optimization (in situ yield of 70%, isolated yield of 45%).

Conclusion

The use of automated equipment coupled with statistical design facilitates the optimization of the acylation reaction. The optimization of the acylation reaction via the conventional method was a time-consuming process, requiring the examination of one reaction variable at a time. Using conventional methods, it took 6 months for two chemists to determine optimal reaction conditions of 3 equiv of $AlCl_3$ in refluxing DCE. Actually, the acylation reaction was evaluated in nitromethane in our previous work; however, the data were not conclusive. With the use of automated equipment coupled with statistical design of experiments, it took only 1.5 months for one chemist to complete the acylation reaction optimization, and the yield of coumarin **3** was greatly improved! Our results demonstrated that automated process research can dramatically accelerate chemical process research and development.

Experimental Section

The reactions were run on a shaker, and the reaction temperature was regulated by a thermocontrol unit. Liquid transfers were conducted with a Gilson 215 liquid handler. Weighing of reaction vials was performed using a Bohdan automated workstation. Samples were dried by using a Savant speed vacuum dryer. The reaction yields were determined in situ with an external standard by HPLC analysis. The HPLC method was validated by Sarawak MediChem Pharmaceuticals, Inc. (column, Chiralpak AD, 250 × 46 mm; mobile phase, 95:5 hexane with 2%TFA: EtOH; flow rate, 1 mL/min; detection, UV 320 nm; sample concentration, 1.00 mg/mL).

Typical Procedure for the Reactions Run under Automated Conditions. Coumarin **3** (250 mg, 1.14 mmol) and Lewis acid (3 equiv) were combined in an 8-mL vial with a permeable cap. DCE (1.5 mL) was added. The resulting mixture was agitated at 65 °C for 15 min, and propionic anhydride (146 μL, 1.0 equiv) in DCE (0.5 mL) was added. The reaction mixture was agitated at 65 °C for 2 h. The warm reaction mixture (100 μL) was transferred to another 8-mL vial containing H_2O (100 μL) and diluted with THF (1.0 mL). The resulting solution was passed through a $MgSO_4$ column, and the organic layer was dried and weighed.

Procedure for the Scale-up Reactions. Coumarin **1** (10 g, 45.4 mmol) and $AlCl_3$ (30 g, 5 equiv) were dissolved in nitromethane (30 mL). The mixture was heated at reflux for 15 min, and a solution of propionic anhydride (5.95 mL, 1.02 equiv) in CH_3NO_2 (10 mL) was added dropwise. The resulting reaction mixture was refluxed for 1 h, after which time HPLC analysis indicated that the reaction was complete (in situ yield 97%). The warm reaction was poured into ice water, and the precipitate was collected by filtration. The precipitates were dissolved in THF. Evaporation of the THF layer afforded crude product as a brown solid (11.4 g, 91%, purity 97% by HPLC analysis).

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