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Process Control Limits from a Laboratory Study on the Ni(0)-Mediated Coupling of Ethyl Acrylate with a C-22 Steroidal Iodide: A Case Study on the Role of Experimental Design in Highly Developed Processes

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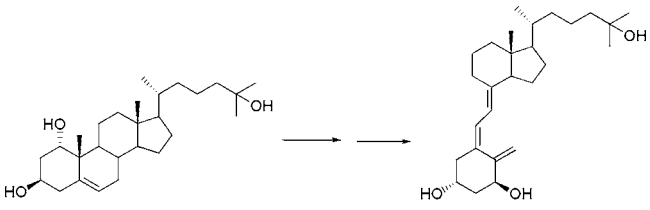
Abstract:

An experimental design was used to assess the process robustness in the Ni(0)-mediated coupling of the C-22 steroidal iodide 15 with ethyl acrylate to yield the coupled product 16. Although the reaction conditions were optimized by empirical means, an experimental design was employed to assess the process sensitivity to certain key factors. Within the experimental space defined by the experimental parameters: moles of ethyl acrylate, moles of water, and moles of nickel chloride hexahydrate, moles of ethyl acrylate was the only significant factor, and no interactive effects were found. The selection of factors was based on mechanistic considerations and from an analysis of competing pathways. The design demonstrated that a potentially capricious reaction was very robust. The predictive equation from the experimental design was used to determine the control limits of the process with respect to the design response, the corrected yield. Process control limits determined from the predictive equation in an experimental design can be used to set realistic process specifications.

Introduction

Statistical design of experiments is an optimization technique which is routinely used in chemical process research and development.[1] In addition to this well-documented application, this technique has recently been used to optimize the reaction conditions for a library synthesis[2] and also to tailor a HPLC analytical method for use in combinatorial synthesis.[3] For chemical processes, the output of such studies is a developed laboratory process in which reaction conditions and processing steps have been well-defined and the rationale for the individual components of the process is understood. When a process is transferred from the laboratory to the pilot plant, an unknown component is the robustness of the process, and although multiple pilot-plant campaigns can be conducted to address this issue, these campaigns can be costly and time-consuming. An alternative way to determine process sensitivity would be to use the predictive equation from an experimental design[4] to predict the control limits of a process.[5] From a consideration of the product-forming and impurity-forming pathways in a nickel-(0)-mediated coupling reaction of a steroidal iodide with ethyl acrylate,[6] a statistical design of experiments was used not only to optimize the reaction conditions but also as a method to evaluate the process sensitivity towards certain key factors.

Scheme 1



1 **2**

Prior to the development of the vitamin D_2-based synthesis of calcitriol (**2**),[6] Roche practiced a synthesis in which one of the key intermediates was 1α,25-dihydroxycholesterol (Ro 21-3245) (**1**)[7,8] (Scheme 1). Starting from 1α,3β-dehydroepiandrosterone (**3**),[9] the synthesis of Ro 21-3245 (**1**) had two critical elements which included the stereospecific introduction of the C-20 methyl group and the elaboration of the

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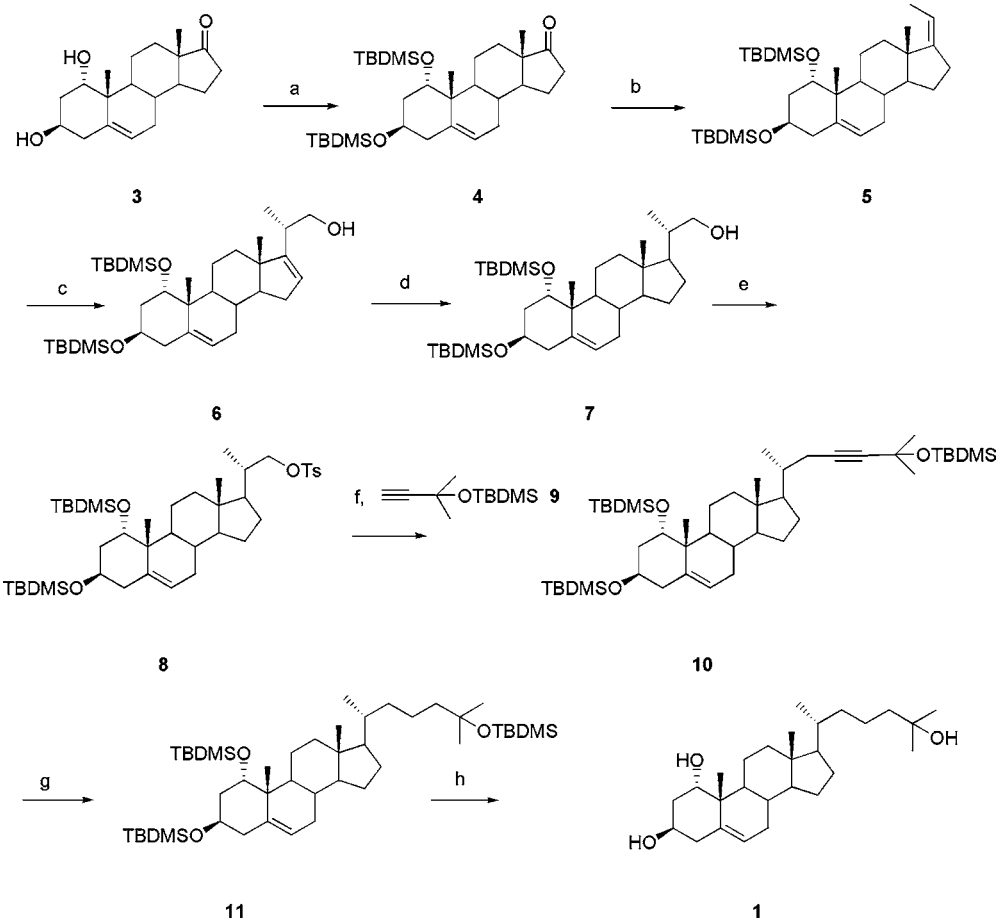
Scheme 2[a]



[a] Conditions: (a) TBDMSCl, imidazole, DMF, 100 °C. (b) $Ph_3PC_2H_5I$, KO*t*-Bu, THF. (c) $(CH_3)_2AlCl$ or $ZnBr_2$, H_2CO, CH_2Cl_2 or hexanes. (d) PtO_2 or Pt/C, EtOAc. (e) *p*-Toluenesulfonyl chloride, pyridine, 0 °C. (f) *n*-BuLi, xylenes, 100 °C (g) Pt/C, EtOAc. (h) $(Bu)_4NF$, THF or DME, reflux.

steroidal side chain.[10] Although this route required the use of the pyrophoric reagent, *n*-butyllithium and the 25-TBDMS-protected alcohol group in the trisilyl ether **11** proved to be difficult to deprotect, this chemistry was successfully executed on a pilot-plant scale (Scheme 2).

Concurrent to the pilot-plant scale production of 1α,25-dihydroxycholesterol (**1**) were activities within the Chemical Synthesis department at Roche for the development of the vitamin D_2-based synthesis of calcitriol (**2**). As described by Manchand and others, a key component of that approach was the Ni(0)-mediated coupling reaction of the C-22 iodide **12** with ethyl acrylate. After thermal deprotection of the triene **13**, a Grignard reaction with methylmagnesium bromide completed the synthesis of the side chain (Scheme 3).[6]

Because of the good yields and the relative ease with which the side chain was constructed, this route was investigated as a substitute for the coupling reaction of the C-22 tosylate **8** with the TBDMS-protected acetylenic alcohol **9** which was currently in production in the pilot plant.[10d,g,h] Because the process to the C-22 tosylate **8** had already been established and quantities were available, the synthesis of the iodide **15** started from the tosylate **8** and not from the alcohol **7**. A Ni(0)-mediated conjugate addition of ethyl acrylate to the C-22 iodide **15** would be followed by a Grignard reaction with methylmagnesium bromide. Deprotection with tetrabutylammonium fluoride would complete the synthesis of Ro 21-3245 (**1**). This synthesis would avoid the preparation of the protected acetylenic alcohol **9** and the difficult deprotection of the TBDMS-protected C-25 group in alcohol **11** (Scheme 4).

The Roche synthesis of 1α,25-dihydroxycholesterol (**1**) started from 1α,3β-dehydroepiandrosterone (Ro 13-1312) (**3**), and the introduction of the 1α-hydroxyl group was accomplished by a fermentation of 3β-dehydroepiandrosterone (**18**) (Scheme 5).[9] As a result, the availability of the starting material for the synthesis of Ro 21-3245 (**1**) was in limited supply. Since the pilot-plant process had already been established for the route based on the coupling of tosylate **8** to the acetylenic ether **9**, any new chemistry for the side-chain construction needed to be reliable and to produce the coupled product **16** in high yield. Because of this, a study of potential process sensitivities and competing pathways for this organometallic coupling reaction was initiated.

Results and Discussion

From a manufacturing perspective, an issue regarding the implementation of this new route for the transformation of

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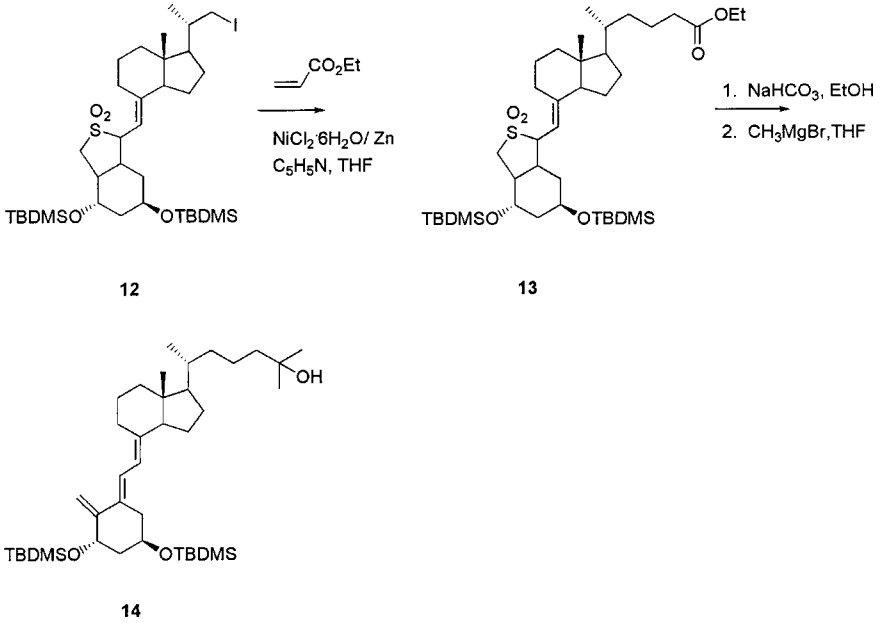
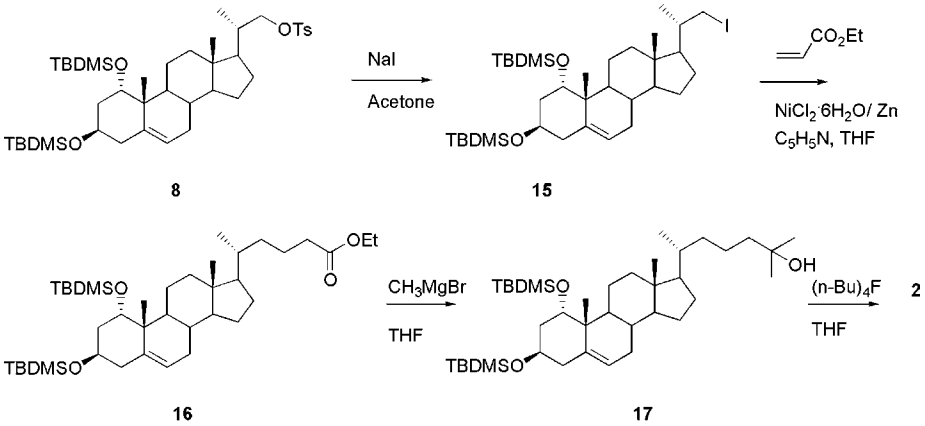
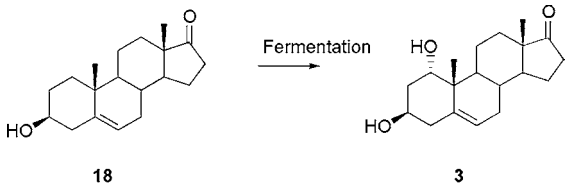
Scheme 3



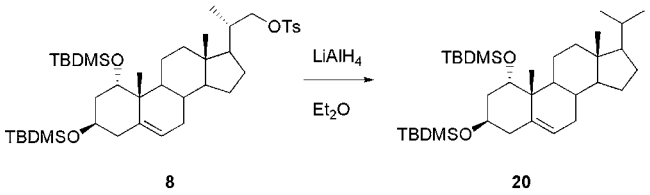
Scheme 4



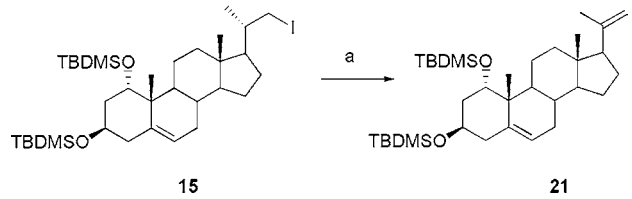
Scheme 5



Scheme 6



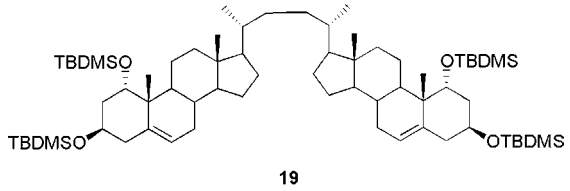
Scheme 7[a]



[a] Conditions: *n*-BuLi, $NiCl_2((Ph)_3P)_2$, $(Ph)_3P$, DBU, THF.

steroidal iodide **15** to the ethyl ester **16** in fixed equipment was the oxygen sensitivity of the Ni(0) complex and its catalytic activity. In particular, laboratory experiments had shown that there was no viable recourse if generation of the catalyst was unsuccessful. Experimental work had also demonstrated that either prolonged reaction times or the addition of more ethyl acrylate would not increase the extent of conversion. Because of this, a process needed to be developed in such a way as to ensure that the activity of catalyst would not be compromised. Preliminary development work had shown that reproducible results could be obtained if both the zinc reducing agent and the nickel chloride hexahydrate catalyst were handled in an inert atmosphere. Despite this important observation, on scale-up, there would be no actual measurement of the oxygen content in the reactor or its contents. Although an inerting procedure would be followed, there would be no specific control over its effectiveness or any additional controls during the transfer of the reagents to the reactor.

In addition to issues regarding catalyst activity, analysis of the crude product indicated the presence of three impurities: a dimeric impurity **19** (Figure 1), a 17-substituted



19

Figure 1.

isopropyl compound **20** (Scheme 6), and a 17-substituted isopropylidiene compound **21** (Scheme 7). The formation of these impurities indicated competing pathways. The dimeric impurity **19** results from a Wurtz-like coupling.[11] The 17-isopropyl substituted steroid **20** forms as a result of hydrolysis of the organonickel complex. Compound **20** was prepared independently by a lithium aluminum hydride reduction of the C-22 tosylate **8**.[12] The 17-isopropylidine steroid **21** results from an elimination of the organonickel complex, and an authentic sample was prepared by the DBU-mediated elimination of an organonickel complex.[13]

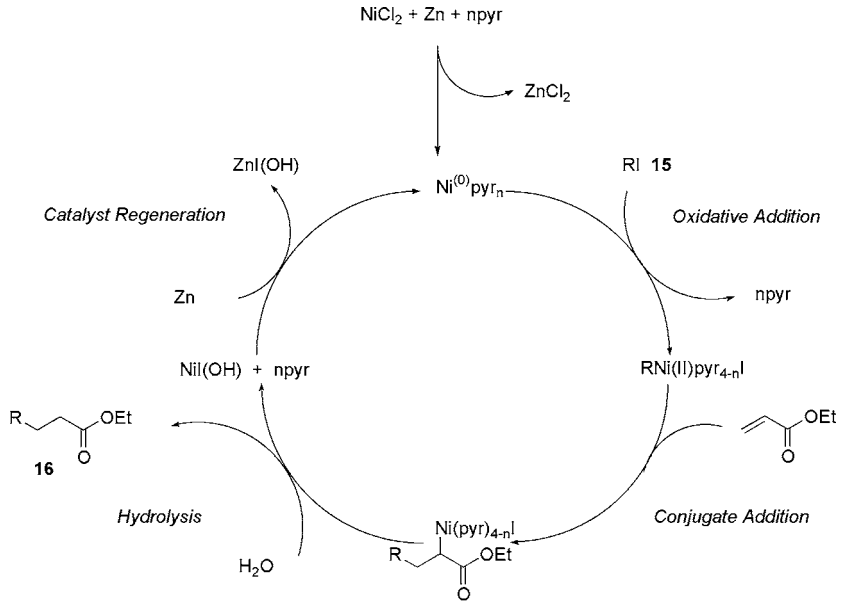
Despite the success with chemistry on a laboratory scale, the process needed to be stressed to understand its response to certain key variables. Our concerns for this process were related to the activity of the catalyst and the expected oxygen sensitivity of the catalyst. Since there was no direct measure of catalyst activity and, more specifically, an in-process control, the lack of this control feature could be justified by an indirect determination of the robustness of the process and by an assessment of the sensitivity of the process to certain key variables. Because of this, an experimental design was used to ascertain the process robustness. The selected variables were concentration of water, concentration of ethyl acrylate, and concentration of nickel chloride hexahydrate. Scheme 8 describes the elementary steps involved in this chemistry.

From a study in a related system, the water content was critical in ensuring high turnover numbers, and on a preparative scale, the use of a 15% molar amount of the hexahydrate of nickel chloride was sufficient.[14] Because the 17-isopropyl steroid **20** was observed as an impurity, there may be an optimum amount of water with respect to the concentration of added water and the concentration of nickel chloride hexahydrate. Because water is required to complete the catalytic cycle, the water concentration may have a pronounced effect on catalyst activity, and this effect might be strongly coupled to the concentration of nickel chloride hexahydrate. In an experimental design, a significant interactive effect between these variables might be observed. Knowledge of this interactive effect may lend insight into potential process sensitivities.

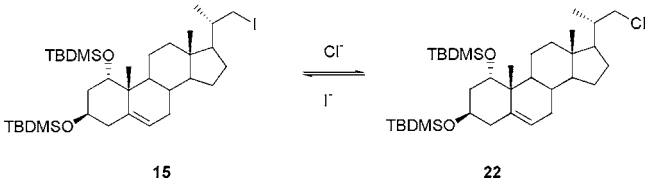
The concentration of ethyl acrylate was expected to be important since the conjugate addition of the organonickel complex with ethyl acrylate is the key carbon−carbon bond-forming reaction. The formation of impurities is indicative of other competing pathways for the organonickel complex. Hydrolysis of the organonickel complex would yield the 17-isopropyl steroid **20**. Coupling of the organonickel complex with the C-22 iodide **15** would yield the dimer **19**. β-Elimination of the organonickel complex would yield the 17-isopropylidene impurity **21**. The presence of an excess of ethyl acrylate would be important since at higher concentrations of ethyl acrylate the rate of the conjugate addition reaction would be increased relative to the rate of impurity formation. Aside from industrial hygiene issues regarding the use of ethyl acrylate, a large excess of ethyl acrylate could increase the rate of polymerization of ethyl acrylate at the expense of the conjugate addition since there is a higher ethyl acrylate rate order dependence for the oligomerization of ethyl acrylate as opposed to the conjugate addition. A second-order ethyl acrylate interactive effect might be expected if the rate of this polymerization reaction were significant.

If the iodide **15** were to be prepared directly from alcohol **7**,[6] iodide **15** may contain triphenylphosphine and experimentally, the level of impurity **21** was increased when triphenylphosphine was present. This effect is presumably

Scheme 8



Scheme 9



15 **22**

related to differences in reactivity of an organonickel compound which is complexed with triphenylphosphine and a complex in which pyridine is the only ligand. The methodology used to synthesize impurity **21** would support this assertation.[13]

The process is catalytic with respect to the nickel chloride hexahydrate concentration. However, from a process perspective, reactions in which there was a reduced amount of nickel would have a greater oxygen sensitivity and this was clearly a situation which was to be avoided. Conversely, owing to the toxicity of nickel compounds, a minimal amount of nickel would be favored for both industrial hygiene and environmental reasons. Higher concentrations of nickel chloride hexahydrate would also increase the likelihood of any Wurtz-type dimerization reaction.

In other development work on the preparation of the C-22 tosylate **8** in pyridine from the C-22 alcohol **7**, the C-22 chloride **22** was observed as an impurity.[15] Because of this, it was feasible that the C-22 chloride **22** could be formed from iodide **15** due to the presence of soluble chloride ion in the coupling reaction and an equilibrium would be established in this exchange (Scheme 9). The reactivity of the chloride **22** with respect to formation of the organonickel complex, the conjugate addition and other competing pathways would be different from the iodide **15**. An optimum amount of nickel chloride hexahydrate might be expected since as the amount of nickel chloride hexahydrate increases so does the amount of soluble chloride.

When we began considering the possibility of using an experimental design to evaluate the process robustness, *in situ* yields were virtually quantitative. Optimal conditions for reaction concentration and temperature for both the preparation of the catalyst and the coupling reaction had been established. Our rationale for using an experimental design was to use this tool to stress the process and to evaluate the process response to certain key variables. An unfamiliarity with the scale-up of processes which utilized this kind of chemistry, the limited supply of the starting material **3**, the multistep process for the synthesis of iodide **15** warranted this detailed study. For this design, the experimental response was the corrected yield.

Our fundamental concern for this process was the reactivity of the zinc reducing agent and the potential oxygen sensitivity of the reaction. In addition to an inertion procedure, other precautions to ensure an oxygen-free reaction were related to manipulations of the zinc and nickel chloride hexahydrate. The zinc and nickel chloride hexahydrate were used as received from Aldrich, and these reagents were handled only in a glovebag. In the pilot plant, a similar operation would be required.

The variables which were investigated were, in part, indirect measures of the reactivity of the catalyst and were designed to assess its reactivity and also the selectivity under stressed conditions. The variables that were evaluated were the molar ratio of added water based on the moles of nickel chloride hexahydrate, the molar ratio of ethyl acrylate based on the moles of C-22 iodide **15**, and moles of nickel chloride hexahydrate based on the moles of C-22 iodide **15**. A 2^3 factorial center composite design was used. The entire factorial experiment was replicated, and the experiments were randomized. The results from the experiment design are listed in Table 1.

The standard protocol involved combining ethyl acrylate, water, nickel chloride hexahydrate, and zinc in a mixture of pyridine and tetrahydrofuran. After heating the mixture to 60−70 °C, the reaction was cooled to 20−25 °C, and a tetrahydrofuran and pyridine solution of the iodide **15** was added slowly. Upon completion of the reaction, the product was isolated. As the focus of the study dealt with different reaction conditions, a product isolation procedure was developed which was nonpractical, but which would ensure that there were no product **16** losses or selectivity with respect to the removal of any impurities. After an area % HPLC analysis of the products, the corrected yields were determined. The corrected yields were in some cases greater than 100% and this was presumably due to the presence of oligomers of ethyl acrylate. The quality of these materials by TLC were very comparable. Comparative TLC was used since the HPLC method did not detect the dimer **19**. A validated weight% percent assay had not been developed when this work was completed and moreover, validation protocols for HPLC methods which utilized evaporative light

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(11) The dimer **19** had the following properties: ^{1}H NMR ($CDCl_3$) δ 0.03 (s, 6H, $-Si(CH_3)_2$), 0.040 (s, 6H, $-Si(CH_3)_2$), 0.049 (s, 6H, $-Si(CH_3)_2$), 0.066 (s, 6H, $-Si(CH_3)_2$), 0.67 (s, 6H, 18-CH_3), 0.88 (s, 36H, $C(CH_3)_3$), 0.93 (s, 6H, 19-CH_3), 2.20−2.22 (m, 4H, 4-CH_2), 3.77 (m, 2H, 1-CH), 5.45 (m, 2H, C=CH). ^{13}C NMR ($CDCl_3$) δ 233.5 Folded over $Si(CH_3)_2$, 235.0 Folded over $Si(CH_3)_2$, 12.0 (C 18), 18.2 ($C(CH_3)_3$), 18.5 (C 19), 18.7 (C 21), 19.4 (C 22), 20.6 (C 11), 26.0 (($C(CH_3)_3$), 28.4 (C 12), 29.8 (C 7), 31.7 (C 7), 32.2 (C 8), 32.2, 36.0 (C 20), 39.0 (C 2), 39.8 (C 16), 41.0 (C 16), 42.2 (C 13), 42.4 (C 4), 56.3 (C 17), 57.1 (C 14), 67.7 (C 1), 73.6 (C 3), 123.1 (C 6), 138.4 (C 5). Exact Mass calcd for $C_{68}H_{126}O_4Si_4$ 1118.87. MS (*m*/*z*) 1118, 1062, 986, 930 (100%), 855, 798, 724.

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(15) The C-22 chloride **22** had the following properties: ^{1}H NMR ($CDCl_3$) δ 0.03 (s, 6H, $-Si(CH_3)_2$), 0.04 (s, 6H, $-Si(CH_3)_2$), 0.05 (s, 3H, $-SiCH_3$), 0.07 (s, 3H, $-SiCH_3$), 0.70 (s, 3H, 18-CH_3), 0.88 (s, 9H, $-(CH_3)_3$), 0.89 (s, 9H, $-(CH_3)_3$), 0.96 (s, 3H, 19-CH_3), 1.10 (d, 3H, 21-CH_3), 2.22 (m, 2H, 4-CH_2), 3.42 (m, 1H, 22′-CH), 3.59 (m, 1H, 22′-CH), 3.77 (m, 1H, 1-CH), 3.99 (m, 1H, 3-CH), 5.45 (m, 1H, C=CH). ^{13}C NMR ($CDCl_3$) δ −4.2 ($Si(CH_3)_2$), −4.5 ($-Si(CH_3)_2$), 12.2 (C 18), 17.6 (C 21), 18.1 (C 23), 19.3 (C 19), 31.6 (C 7), 38.9 (C 2), 42.4 (C 4).

Table 1. **Statistical design of experiments for the coupling of iodide 15 with ethyl acrylate**

experiment	added water[a]	ethyl acrylate[b]	nickel chloride hexahydrate[c]	corrected yield (%) of ethyl ester **16**[d]
1	6	2.2	0.25	105.2
				100.6
2	3	2.2	0.25	95.6
				100.2
3	6	2.2	0.125	102.8
				96.0
4	3	2.2	0.125	99.2
				95.8
5	6	1.5	0.25	94.3
				96.2
6	3	1.5	0.25	93.9
				98.3
7	6	1.5	0.125	97.6
				97.1
8	3	1.5	0.125	95.0
				96.7
center point	4.5	1.8	0.188	96.3
				98.7

[a] Molar ratio based on the moles of added water and the moles of nickel chloride hexahydrate. [b] Molar ratio based on the moles of ethyl acrylate and the moles of the iodide **15**. [c] Molar ratio based on the moles of nickel chloride hexahydrate and the moles of the iodide **15**. [d] The corrected yield is equal to the actual weight yield times the HPLC area percent assay.

scattering as the detection method had not yet been established.

Using the program D.O.E. fusion,[16] the data was analyzed, and the only significant variable was the concentration of ethyl acrylate. None of the other variables or any interactive effects were significant relative to the experimental error. The predictive equation from this experimental design is defined by the following expression: corrected yield = 97.9 + 4.33[ethyl acrylate].

The observation that neither the moles of nickel chloride hexahydrate or the moles of added water were significant indicates that the catalyst remains very active despite the fact that the design explored a fairly comprehensive experimental space. Earlier optimization experiments had identified appropriate reaction conditions and the inertion procedure was sufficient in excluding oxygen from the reaction. Indeed, this design showed that the process is very robust.

Because the process is now defined by a predictive equation, this equation was used to predict the control limits of the process. Using the random number generator feature in Excel, values of the concentration of ethyl acrylate were allowed to vary from 1.85 ± 0.0925. This variation would be considered as a typical allowable tolerance limit for a reagent in a manufacturing environment. Using the predictive equation from the experiment design, the control chart limits for this process using a subgroup of one was calculated. On the basis of this, the corrected yield in this process would be expected to vary from 100.40% to 100.95%. In addition to its use as a way to optimize processes and assess process variability, the predictive equation from an experimental design can also be used to set specifications for a given process attribute. Realistic process specifications would then be set at values greater than the upper control limit and less than the lower control limit.

For use during piloting, this laboratory development work and analysis would be helpful to determine if the plant process is different than that which was described by the laboratory model. Corrected yields which were outside of the control limits would indicate that the variation which was observed in the laboratory model is different than the plant process. The preparation and the *in situ* regeneration of the Ni(0) catalyst is a heterogeneous reaction whereas the conjugate addition is a homogeneous reaction. For such a situation, a reasonable explanation would be that the sensitivity of the laboratory process to mass transfer effects is different than in the plant setting. Analysis of the final product **16** for residual starting material **15** may support such a conclusion. Since the laboratory model was optimized to minimize the formations of impurities **19**, **20**, and **21**, quantification of the amounts of these impurities would be very informative. As the formation of these impurities would be increased if the amount of ethyl acrylate was somehow reduced, this analysis would pinpoint the differences between the laboratory model and a pilot-plant campaign.

Conclusions

An experimental design was used to assess the process robustness for the transformation of steroidal iodide **15** to the ethyl ester **16**. Although the reaction conditions were optimized by empirical methods, an experimental design was found to be very useful in assessing the robustness of a process. In addition, the predictive equation from the experimental design was used to determine the control limits of the process with respect to the design response, the corrected yield. These control limits can be used to set process specifications.

Experimental Section

The NMR spectra were obtained using a Varian Gemini 200 MHz spectrophotometer, a Varian Unity 400 MHz spectrophotometer, or a Varian 400 FT-MHz spectrophotometer. All spectra are referenced to tetramethylsilane unless otherwise noted. IR spectra were obtained on an Analect FX 6260 FT-IR spectrophotometer. The tetrahydrofuran and pyridine were obtained from Fisher Scientific. The ethyl acrylate, zinc dust, and nickel chloride hexahydrate were obtained from Aldrich Chemical Co.

HPLC Conditions. The HPLC conditions which were used consist of the following: a Zorbax ODS 25.0 cm × 4.6 mm column was used with an eluent that consisted of a mixture of 65% acetonitrile and 35% methylene chloride. The flow rate was 0.8 mL/min. A Varex evaporative light scattering detector (ELSD II) (operating at an exhaust temperature of 40 °C, a heater temperature of 49 °C, and a gas flow of 60 mm, 21 psig) was used. Table 2 lists the retention times of the starting material **15**, impurities, and final product **16**.

Typical Coupling Procedure: Preparation of Ro 25-1074 (16). Under a nitrogen atmosphere were combined 6

(16) D.O.E. Fusion is available from S-Matrix, Corp.; 835 Third Street; Eureka, CA 95501.

Table 2. **HPLC retention times**

component	retention time (min)
Ro 25-1074 (**16**)	16.9
Ro 25-0232 (**15**)	18.4
Ro 25-0369 (**21**)	20.2
Ro 25-0368 (**20**)	29.2

mL of tetrahydrofuran, 6 mL (0.074 mol) of pyridine, 2.55 mL (0.023 mol) of ethyl acrylate, 0.19 mL (0.011 mol) of water, 3.63 g (0.056 mol) of zinc, and 0.57 g (0.0024 mol) of nickel chloride hexahydrate. Agitation by means of a mechanical stirrer was started, and the batch was heated between 60 and 70 °C for 30 min. The temperature reached 60 °C in 3.75 min. The heat was removed, and the batch was cooled to 24.5 °C over 23 min. A solution of the 8.80 g (0.028 mol) of the C-22 iodide **15** in 16 mL of tetrahydrofuran and 6 mL of pyridine was added over 96 min. The solution was prefiltered through Celite. A sample was removed for HPLC analysis 5 min after the addition was complete, and another sample was removed 1 h and 56 min later. After the addition was complete, the reaction was quenched 2 h and 37 min later by pouring the reaction into 350 mL of acetone. The reaction was filtered, and the filtrate was poured into 500 mL of water. The batch was stirred for 2.5 h at 13−23 °C. The batch was filtered, the cake was washed with water, and the solid was then washed with a mixture of water and methanol. The solid was dried overnight at 34−35 °C under vacuum. There was obtained 8.16 g of the ester Ro 25-1074 (**16**) as a white powder (96.3%).[6b]

The ester **16** had ^{1}H NMR ($CDCl_3$) δ 0.028 (s, 3H, $-SiCH_3$, 0.040 (s, 3H, $-SiCH_3$), 0.048 (s, 3H, $-SiCH_3$), 0.067 (s, 3H, $-SiCH_3$), 0.67 (s, 3H, 18-CH_3) 0.88 (s, 18H, 2 × $-C(CH_3)_3$), 1.25 (t, 3H, CH_3), 2.25 (m, 4H, 4-CH_2, $-CH_2C$═O), 3.77 (m, 1H, 1-CH), 3.97 (m, 1H, 3-CH), 4.12 (q, 2H, $-OCH_2$), 5.45 (m, 1H, C═CH). ^{13}C NMR ($CDCl_3$) δ −5.2 ($-SiCH_3$), −4.6 ($-SiCH_3$), −4.4($-SiCH_3$), −3.7($-SiCH_3$), 12.0 (C 18), 14.3 (C 27), 18.1 (C 28), 18.2 (C 34), 18.6 (C 21), 19.3 (C 19), 20.6 (C 11), 21.6 (C 23), 24.4 (C 15), 26.0 (2 × $-(CH_3)_3$), 28.3 (C 12), 31.6 (C 7), 32.1 (C 8), 34.8 (C 20), 35.4 (C 22), 35.6 (C 24), 39.0 (C 2), 39.7 (C 4), 40.9 (C 9), 42.2 (C 13), 42.4 (C 10), 55.9 (C 17), 57.0 (C 14), 60.1 (C 26), 67.6 (C 1), 73.6 (C 3), 123.3 (C 6), 138.4 (C 5), 173.9 (C 25). IR (KBr) (cm^{-1}) 2954 (s), 2934 (s), 1740 (m, C═O), 1256 (m, Si−O), 1095 (m, C−O), 1076 (m, C−O).

The final products from the experimental design were analyzed by comparative TLC. The stationary phase was silica gel, and a mixture of 75% hexane and 25% ethyl acetate was used as the eluent. Standard solutions from each experiment were prepared, and 20 μL of each sample was spotted. The plate was visualized with 5% phosphomolybdic acid in ethanol. By visual inspection, no differences were observed in these samples.

Preparation of (1α,3*β*)-20S-Methyl-1,3-bis[[(1,1-dimethylethyl)dimethylsilyloxy]pregn-5-ene-22-iodide (Ro 25-0232) (15). Ro 24-7561 (**8**) (421 g, 0.576 mol), sodium iodide (604 g 4.03 mol), and 10 L of acetone were combined.[10h] The batch was heated to reflux and held there for 2.5 h. The batch was cooled to 0−5 °C, and a solution of 229.6 g (0.925 mol) of sodium thiosulfate pentahydrate in 2 L of water was added. During the addition, the temperature rose to 36 °C, and the batch was allowed to stir at ambient temperature overnight. To the reaction, 11 L of ice water was added, and the batch was stirred at ambient temperature for 2.5 h. The batch was filtered, and the cake was washed with 2 × 2L of water. The cake was dried on a funnel and then in a vacuum oven at 65−70 °C. There was obtained 388.7 g of Ro 25-0232 (**15**) as a white powder. The solid was recrystallized from 5.81 L of methanol. There was obtained 349 g of Ro 25-0232 (**15**) as a white powder. (88.2% yield). An analytical sample had the following properties: mp 145−147 °C. Anal. Calcd for $C_{34}H_{63}IO_2Si_2$ (686.95) C, 59.45; H, 9.24; I, 18.34. Found: C, 59.60; H, 9.23; I, 18.69. $[\alpha]^{22} = -22.83$ (c =1.0%, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ 0.03 (s, 6H, $-SiCH_3$), 0.05 (s, 3H, $-SiCH_3$, 0.07 (s, 3H, $-SiCH_3$, 0.71 (s, 3H, 18-CH_3), 0.88 (s, 9H, $-C(CH_3)_3$), 0.89 (s, 9H, $-C(CH_3)_3$), 0.96 (s, 3H, 19-CH_3), 1.02−1.04 (d, 3H, 21-CH_3), 1.83−1.94 (m, 4H, H-2, H-7), 2.18−2.32 (m, 2H, CH_2), 3.16 (m, 1H, H-22′) 3.32−3.35 (m, 1H, H-22′), 3.35(m, 1H, H-1), 3.76 (m, 1H, H-3), 5.44 (m, 1H, C═CH). ^{13}C NMR ($CDCl_3$) δ −5.1 ($-SiCH_3$), −4.5 ($-SiCH_3$), −4.4 ($-SiCH_3$), −3.8 ($-SiCH_3$), 12.7 (C 18), 18.1 (C 23), 19.3 (C 19), 20.5 (C 21), 20.8 (C 22), 20.9 (C 11), 24.3 (C 15), 25.8 ($-(CH_3)_3$), 26.0 ($-(CH_3)_3$), 27.6 (C 12), 31.6 (C 7), 32.2 (C 8), 37.1 (C 20), 38.9 (C 2), 39.4 (C 13), 40.8 (C 16), 42.2 (C 4), 42.3 (C 9), 42.4 (C 10), 55.5 (C 17), 56.5 (C 14), 67.5 (C 1), 73.5 (C 3), 123.1 (C 6), 138.4 (C 5). IR (KBr) cm^{-1} 1672 (w, C═C), 832 (m, C−I)

Preparation of (1α,3*β*)-20-Methyl-1,3-bis[(1,1-dimethylethyl)dimethylsilyoxy]pregna-5-ene (Ro 25-0368) (20). Under a nitrogen atmosphere, a solution of 4.67 g (6.4 mmol) of Ro 24-7561 (**8**) in 26 mL of diethyl ether was prepared. A total of 0.52 g (13.7 mmol) of lithium aluminum hydride in portions was added, and the batch was stirred for approximately 1.5 h.[12] Water (5 mL) was added, and the batch was stirred until hydrogen evolution ceased. The batch was filtered, and the cake was washed with diethyl ether. Saturated sodium bicarbonate solution (20 mL) was added. The layers were separated, and the organic layer was dried over sodium sulfate. Solvent was removed under vacuum, and the residue was recrystallized from 50 mL of methanol. There was obtained 2.56 g of the 17-isopropyl compound **20** as a white solid in a 71% yield. Ro 25-0368 (**20**) had mp 161−162 °C; Anal. Calcd for $C_{34}H_{64}O_2Si_2$ (562.03) C, 72.79; H, 11.50; Found C, 72.81; H, 11.53. $[\alpha]^{25} = {}^{+}6.29$ (c = 1.0%, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.028 (s, 3H $-SiCH_3$), 0.039 (s, 3H, $-SiCH_3$), 0.048, (s, 3H, $-SiCH_3$) 0.067 (s, 3H, $-SiCH_3$), 0.67 (s, 3H 18-CH_3), 0.83 (d, 3H, 21-CH_3), 0.88 (s, 18H, 2 × $-(CH_3)_3$), 0.93 (m, 3H, 20-CH_3), 0.96 (s, 3H, 19-CH_3), 2.25 (m, 4H, 20-CH, 21-CH_3), 3.76 (m, 1H, 1-H), 3.98 (m, 1H, 3-H), 5.45 (m, C═CH). ^{13}C NMR ($CDCl_3$) δ −5.2 ($-SiCH_3$), −4.5 ($-SiCH_3$), −4.4 ($-SiCH_3$), −3.7 ($-SiCH_3$), 12.1 (C 18), 18.1 (C 23), 18.2 (C 29), 19.4 (C 19), 20.5 (C 11), 22.5 (C 21), 23.2 (C 22), 24.4 (C 15), 25.0 ($-(CH_3)_3$), 25.04 ($-SiC(CH_3)_3$), 28.5 (C 12), 31.1 (C 7), 31.7 (C 8), 32.1 (C 20), 39.0 (C 2), 39.6 (C 10), 40.9 (C

16), 42.2 (C 13), 42.4 (C 9, C 4), 57.0 (C 14), 58.3 (C 17), 67.6 (C 1), 73.6 (C 3), 123.3 (C 6), 138.4 (C 5).

Preparation of (1α,3β)-1,3-Bis[(1,1-dimethylethyl)dimethylsilyoxy]-20-methylpregna-5,20-diene (Ro 25-0369) (21). To 3.27 g (5 mol) of dichlorobis(triphenylphosphine)-nickel(II) in 200 mL of tetrahydrofuran was added 7.87 g (30 mmol) of triphenylphosphine. A 1.6 M solution of *n*-butyllithium (3.5 mL) was added, and the solution turned from green to a reddish-brown. To the reaction was added a solution of 3.43 g (5.0 mol) of Ro 25-0232 (**15**) and 1.5 mL (10.0 mol) of DBU in 30 mL of tetrahydrofuran.[13] The solution was stirred overnight at room temperature. The reaction was quenched by the addition of 50 mL of 2 N hydrochloric acid and 150 mL of hexane. The batch was filtered, and the organic layer was separated. The organic layer was washed with 50 mL of saturated sodium bicarbonate solution and 50 mL of saturated sodium chloride solution. The organic layer was dried over sodium sulfate, and the solvent was evaporated. To the solid was added 50 mL of hexane, and the batch was cooled in an ice bath. The resulting solid, triphenylphosphine, was removed by filtration, and the solid was evaporated. The residue was recrystallized from 30 mL of methanol. There was obtained 1.70 g of the 17-isopropylidene steroid **21** in a 61% yield. Ro 25-0369 (**21**) had mp 155−157 °C. $[\alpha]^{RT} = -154.4$ °C (1.0% in $CHCl_3$). Anal. Calcd for $C_{34}H_{62}O_2Si_2$ (559.05) C, 73.05; H, 11.18. Found C, 72.76; H, 11.35. ^{1}H NMR ($CDCl_3$) δ 0.042 (s, 6H, $-SiCH_3$), 0.052 (s, 3H, $-SiCH_3$), 0.074 (s, 3H, $-SiCH_3$), 0.58 (s, 3H, 18-CH_3), 0.88 (s, 9H, $-C(CH_3)_3$), 0.94 (s, 9H, $-C(CH_3)_3$), 0.97 (s, 3H, 19-CH_3), 1.73−1.76 (m, 3H, 21-CH_3), 2.26−2.32 (m, 2H, 4-CH_2), 3.78−3.95 (m, 1H, 1-CH), 3.96−4.02 (m, 1H, 3-CH), 4.71 (s, 1H, 22′-CH), 4.85 (s, 1H, 22′-CH), 5.45−5.47 (m, 1H, C=CH). ^{13}C NMR ($CDCl_3$) δ −5.1 ($-SiCH_3$), −4.8 ($-SiCH_3$), −4.4($-SiCH_3$), −3.7($-SiCH_3$), 12.9 (C 18), 18.2 (C 23, C 29), 19.4 (C 19), 20.6 (C 11), 24.4 (C 21), 24.6 (C 15), 25.5 (C 12), 25.9 ($-(CH_3)_3$), 31.6 (C 7), 32.5 (C 8), 38.6 (C 4), 39.0 (C 2), 41.2 (C 16), 42.3 (C 9), 42.4 (C 10), 43.2 (C 13), 56.8 (C 14), 57.4 (C 17), 67.6 (C 1), 73.7 (C 3), 110.7 (C 22), 123.2 (C 6), 138.6 (C 5), 145.7 (C 20). IR (KBr) (cm^{-1}) 2955 (s), 1641 (w, C=C), 1256 (m, Si−O), 1095 (s, C−O), 1075 (s, C−O).

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