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Process Development and Scale-Up of an Hsp90 Inhibitor

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Supporting Information

ABSTRACT: A scalable process for the manufacture of a Hsp90 inhibitor was developed and optimized. Key features in the seven-step process include a selective S_NAr reaction followed by an Ullmann-type coupling of indazolone to an aryl halide. This improved process afforded 65% yield over two critical steps compared to 25% following the Medicinal Chemistry route.

INTRODUCTION

SNX-5422 (**11**) belongs to a novel class of heat shock protein 90 (Hsp90) inhibitors,[1] which have recently attracted much interest as a therapeutic target for cancer treatment. This compound is one such potential therapeutic agent, currently in phase II clinical studies. The medicinal chemistry route[2] to the synthesis of this active pharmaceutical ingredient (API) is outlined in Scheme 1. This synthesis was used to prepare tens of grams for preclinical studies. The two main structural components for building the core structure were **3** and **6** which were coupled in dimethylacetamide (DMAc) at high temperatures to afford **7** which was then hydrolyzed to **8** and then coupled with Boc-glycine followed by deprotection to give **10**. The freebase was then converted to the mesylate salt to afford the API.

Our initial assessment of this route for larger-scale synthesis pointed out several issues. The coupling of **1** and **2** resulted in the formation of two regioisomers, **3** and **3a** (1:3), in favor of the undesired isomer. The separation of the desired product was accomplished by repeated column chromatography on silica gel as the two products eluted very closely. Coupling of tosylhydrazide with dimedone was conducted in toluene which was not polar enough to maintain a stirrable slurry. In trial reactions, the product **4** formed a thick unstirrable mixture. Conversion of **4** to indazolone **6** gave varying results and often led to isolation issues and poor yields (30−35%). Another major concern was that the thermal coupling of **3** and **6** was carried out at 150 °C using sodium hydride in dimethylacetamide and posed a potential hazard on scale. Furthermore, the product was found to be unstable at such temperatures. Compound **10** was found to be poorly soluble in most organic solvents, and hence a methanol/methylene chloride solvent combination was used to prepare the mesylate salt, thereby raising concerns of genotoxic impurities and waste disposal issues for methylene chloride. Our new process addresses successfully all of these concerns.

RESULTS AND DISCUSSION

Since most of the major issues were in the early steps of the synthesis, our initial efforts focused on optimizing the preparation of **3** following the Medicinal Chemistry route. Screening of different bases, solvents, and temperatures resulted only in marginal improvement of the ratio of the two isomers. Neither could we develop a nonchromatographic separation of the two isomers that could be used in large-scale synthesis. Failing in this endeavor, our development now concentrated on evaluating other starting materials that could selectively give the desired product.

Synthesis of Coupling Partners 13 and 6. It was thus important to differentiate the two reactive positions to improve the selectivity. On the basis of commercial availability and price, our initial assessment was done on 2-bromo (or chloro)-4-fluorobenzonitrile to first selectively displace the bromide under palladium-catalyzed conditions. Although these conditions were moderately successful, we quickly realized that such a transformation was not cost-effective which often resulted in poor yields and involved expensive catalysts.[3] We then turned towards 4-bromo-2-fluorobenzonitrile **12** (Scheme 2) as the key starting material toward intermediate **7**

Scheme 2 outlines the second approach. The coupling of **13** and **6** could conceivably be done using copper-mediated Ullmann-type[6] coupling. Compound **13** could potentially be obtained from the thermal coupling of 4-bromo-2-fluorobenzonitrile with 4-aminocyclohexanol. Our rationale for using **13** for the Ullmann coupling was based on consideration of electronic factors. The presence of the amine group offsets the electron-withdrawing effect of the nitrile and facilitates the copper-mediated coupling compared to first installing the indazolone **6**, where the aryl halide is a very electron-deficient system.

4-Bromo-2-fluorobenzonitrile **12** was a useful substrate in that the first displacement (S_NAr) could be done on the more reactive fluorine selectively. We carried out the first displacement under previously used conditions in DMSO at 125 °C. Gratifyingly, the reaction with 4-bromo-2-fluorobenzonitrile gave exclusively the desired isomer derived from fluoride displacement. The alternative, potential product derived from the displacement of the bromide was not detected.

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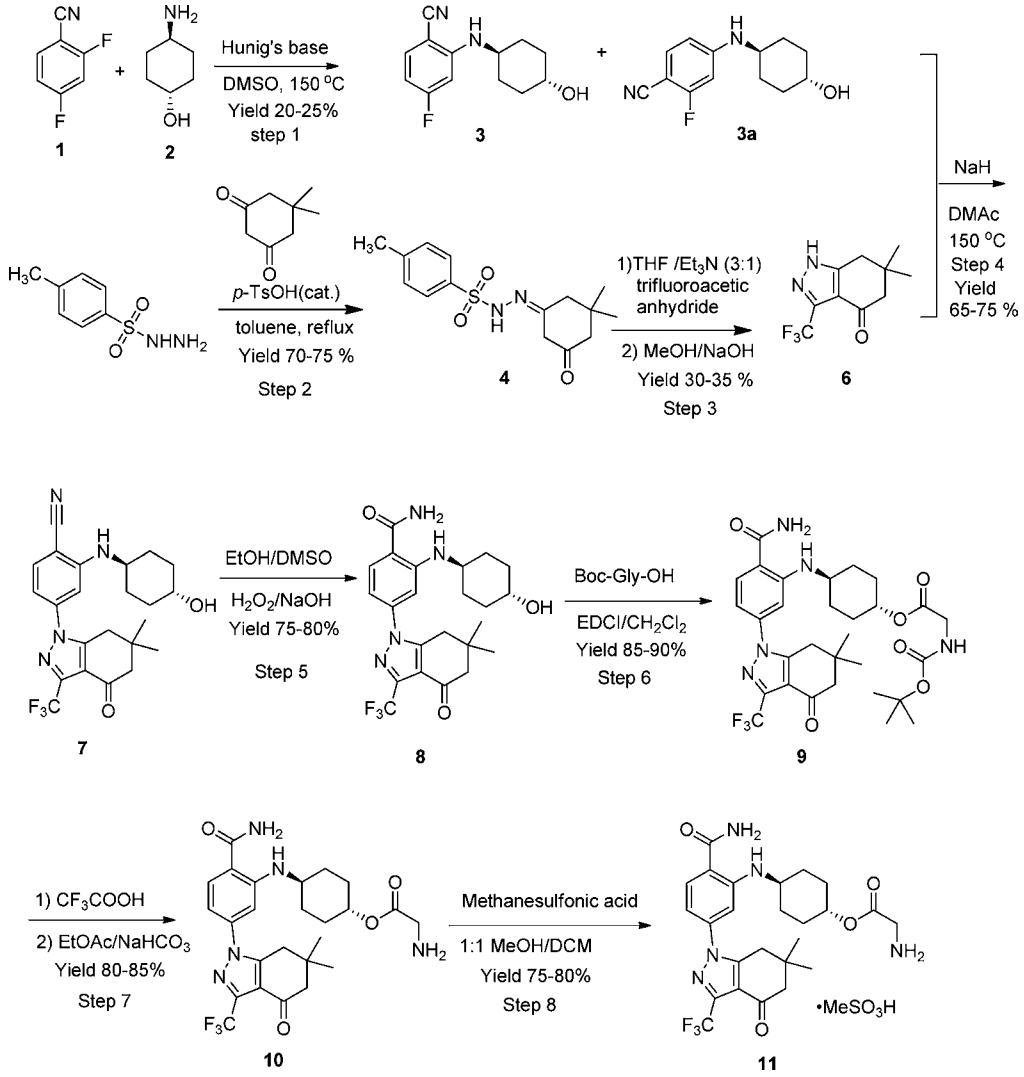
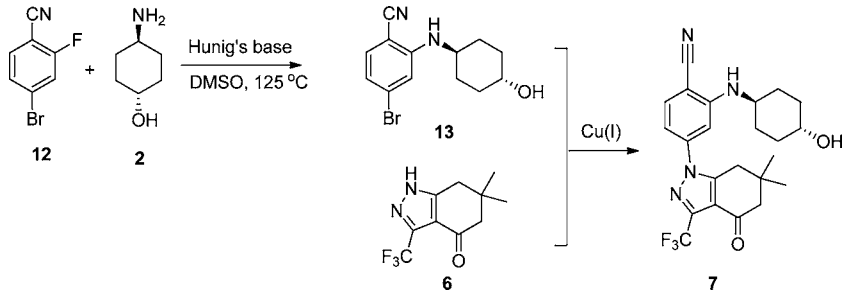
Scheme 1. Medicinal Chemistry synthesis of 11



Scheme 2. Alternative chemistry via Ullmann-type coupling



Running high-temperature reactions using DMSO at production scale is not preferred due to the possible decomposition of DMSO. In this case we conducted a stability assessment of the reaction mixture,[4] and no thermal events were observed upon heating the reaction mixture to 200 °C. Our safety group considered it safe to operate 75 °C below this temperature.[5] An aqueous workup, followed by extraction of the product with ethyl acetate and then crystallization, consistently afforded the coupling product **13** in 85−90% yields and >99% (AUC) purity.

The synthesis of indazolone **6** proceeds through the formation of tosylhydrazone[7] **4** (Scheme 3). This condensation reaction of tosylhydrazide and dimedone was originally conducted in toluene.

The product precipitated out during the reaction, and this phase separation assisted in driving the reaction to completion; thus, water did not need to be removed to drive the equilibrium. However, the bulk of the product precipitated all at once and formed a thick, unstirrable mixture although there were 40 volumes of solvent. This was solved by using a more polar solvent (THF) instead, as it permitted a gradual

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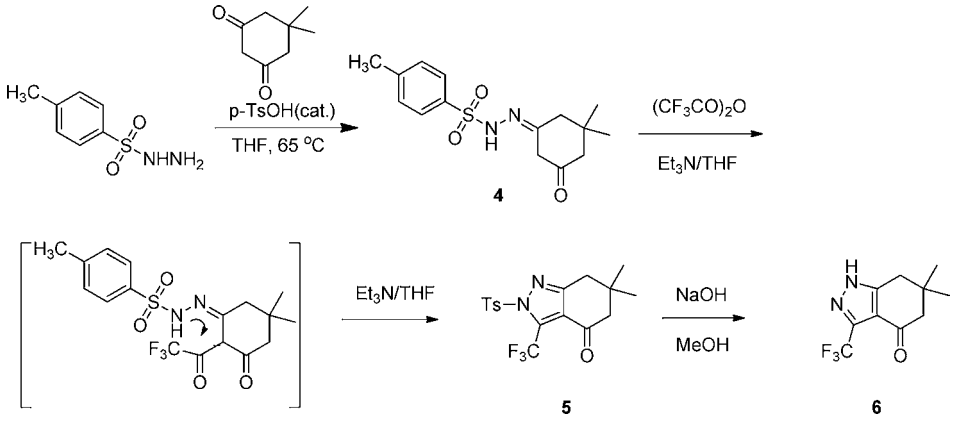
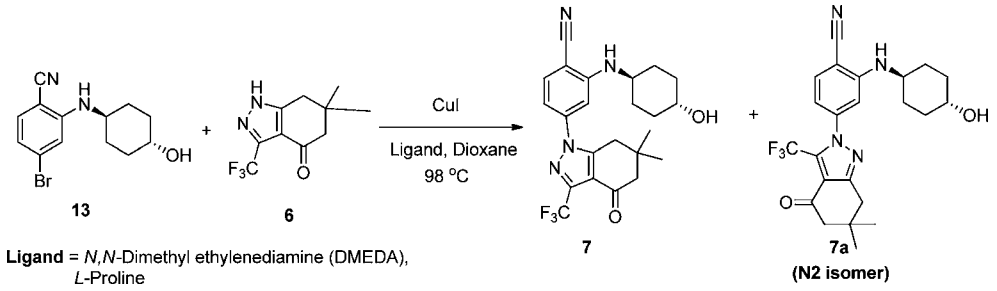
Scheme 3. Formation of indazolone 6



Scheme 4. Copper-mediated Ullmann coupling



precipitation due to higher solubility that was easy to stir and filter. The main impurity was the unreacted starting material which stayed in solution. The product was readily filtered and washed to afford material in 80% yield and >99% purity.

The preparation of indazolone **6** involved the trifluoroacetylation of compound **4** via addition of trifluoroacetic anhydride (TFAA) in the presence of excess triethylamine followed by cyclization of the derived intermediate. The reaction proceeds through an initial formation of acylated intermediate[8] which then cyclizes to form the tosyl indazolone **5** (Scheme 3). Intermediate **5** was then converted to **6** under basic conditions.

Due to varying levels of impurities, we carried out two control experiments each in the absence of either triethylamine or trifluoroacetic anhydride. The reaction conducted in the absence of TFAA showed significant amounts of one of the major impurities. The structure of this impurity was consistent with the structure of a dimer of **4**,[9] presumably derived from an aldol self-condensation of **4** under the strongly basic conditions. On the other hand, no degradation or generation of impurities was observed in the absence of triethylamine. These observations helped rationalize the lower-purity profile for reactions conducted in an initially basic medium (Et_3N/THF) with an extended TFAA addition time. Yet another critical parameter was the reaction temperature which had to be maintained above 35 °C. Lower temperatures led to significant decomposition due to the lack of sufficient energy of activation for the cyclization to occur.[10] A calorimetric assessment (RC1) carried out indicated the heat of the reaction (ΔH_{rxn}) was 250 KJ/mol and ΔT_{ad} was 51 °C which equated to a maximum temperature of synthetic reaction (MTSR) of 91 °C, higher than the boiling point of the solvent. The heat release was directly proportional to the addition rate of triethylamine. Thus, the process was redesigned such that compound **4** was first reacted with TFAA, and then triethylamine was added while maintaining the reaction temperature between 35 and 45 °C. This process change resulted in an improved synthesis of **5** with impurity levels at <2%. The tosyl cleavage of the intermediate **5** was carried out in the same pot by simply adding sodium hydroxide solution at 60 °C. This conversion was very robust and resulted in a clean reaction profile. The product was isolated by crystallization from ethyl acetate/heptanes in 60% yield and >99% purity compared to 30−35% following the original process.

Ullmann Coupling of 13 and 6. There are several reported examples of copper-catalyzed coupling of aryl bromides with pyrroles, pyrazoles, indazoles, imidazoles, and triazoles catalyzed by copper−diamine complexes.[11] Ma[12] has also reported an Ullmann-type coupling of amines with aryl halides using proline or methyl glycine as ligands for the copper-catalyzed coupling reactions. We were very interested in applying this chemistry for our coupling of **13** and **6** (Scheme 4).

We first screened two reported ligands, Ligand A (*N*,*N*-dimethyl ethylenediamine) and Ligand B (L-proline) as these two ligands were commercially available in large quantities and are inexpensive. Potassium carbonate was used as the base in all the cases, and the reactions were run at 98−100 °C (Table 1).

Table 1. Screening conditions for the Ullmann coupling

solvent	ligand	conversion (% area, 24 h)
toluene	A	20
dioxane	A	25
DMSO	B	15
degassed dioxane	A	50
10% aqueous dioxane	A	60

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On the basis of these initial screening results after 24 h, degassed dioxane was chosen as the solvent for further optimization. Although the conversion was higher in 10% aqueous dioxane, almost 15% of the unwanted N2 regioisomer was formed which did not purge well in downstream chemistry. This impurity had to be limited to <1% at this stage to completely eliminate the corresponding derivative in the API (Figure 1). By running the reactions in degassed dioxane longer

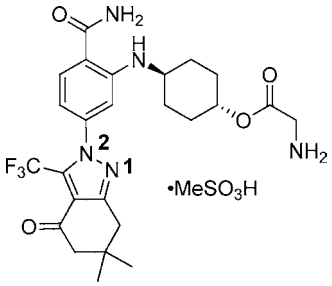

Figure 1. N2 impurity in the API.

(60−65 h) it was possible to achieve complete conversion with 2−4% of the N2 isomer. The kinetics could not be significantly improved under pressure at higher temperatures. Other bases such as KH_2PO_4 or Cs_2CO_3 yielded similar results. Hence, the reaction was optimized to run at 98 °C in degassed dioxane. Degassing the solution reduced deactivation of the catalyst as evidenced by a green color formation typical of the oxidized Cu(II) species in incomplete reactions. Isolation involved quenching with ammonium hydroxide to solubilize the copper salts, extraction using ethyl acetate, and crystallization by addition of heptanes. The isolated solids still contained 1.0−1.5% of the N2 isomer. Altering the cooling profile or reducing the volumes of the antisolvent during isolation did not reduce the N2 isomer; hence, a hot reslurry of crude **7** in ethyl acetate was developed which consistently reduced this impurity to <0.5% in an overall 70−75% yield with 8−10% loss in the mother liquors.

Nitrile Hydrolysis of 7. The subsequent hydrolysis of the nitrile **7** to the amide **8** was conducted in ethanol/DMSO mixture using sodium hydroxide and hydrogen peroxide[13] as catalyst. The original small-scale all-in-one batch process created a large exotherm and was not considered safe for large-scale production. Calorimetric screening (RC1) indicated that the heat of the reaction was 371 kJ/mol, corresponding to a theoretical adiabatic temperature rise of 40.7 °C. The heat output of the reaction was also found to be proportional to the addition rate of hydrogen peroxide. Thus, the process was modified to add hydrogen peroxide over an extended time to control the reaction exotherm. The initially developed isolation procedure for **7** following an aqueous quench and extractive workup using ethyl acetate was not very robust. Successful crystallization depended on the amount of impurities, water content, and rate of addition of the antisolvent. No reduction of the N2 isomer derivative was seen in this crystallization. Further evaluation led to the development of a direct isolation procedure by adding water to the reaction mixture. By optimizing the amount of water added to the batch to induce crystallization, both the mass recovery and the product purity were readily controlled and maximized.

Glycine Ester Formation. The ester formation using Boc-glycine was carried out in methylene chloride using EDC as the coupling reagent. The main impurity generated in this reaction was an overacylated product[14] (Figure 2). Very interestingly, when Boc-glycine was stirred with EDC and DMAP in the

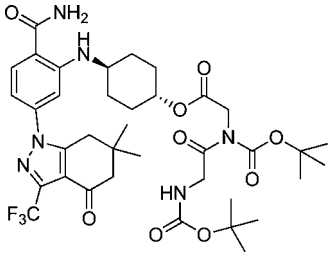

Figure 2. Impurity 2.

absence of the coupling partner, compound **8**, the reaction mixture was found to contain approximately 50% of Boc(Gly)-Boc(Gly)−OH. Under the reaction conditions small amounts of Boc(Gly)Boc(Gly)−OH are formed which then couple with compound **8**, giving rise to this impurity at 0.5−1.0% levels. Temperature dependence of the impurity pointed out that higher amounts were formed even with marginal increase in reaction temperature. Running the reaction at 30 °C increased the impurity level to >2%. No significant drop in levels was observed when the reaction temperature was lowered, although the reaction took longer to reach completion. It was essential to control this impurity at this step to <0.35% to limit the corresponding deprotected derivative in the isolated API to less than 0.25%, which was the acceptable limit based on the qualification level in toxicology studies.

Extraction of the product **9** into ethyl acetate and crystallization upon addition of heptanes could not reduce this impurity to below the 0.25% threshold. Thus, a separate purification step was needed to upgrade the purity of the product. This was achieved by reslurry in hot ethyl acetate which was very effective in reducing this impurity to acceptable levels.

One-Pot Deprotection and Mesylate Salt Formation. The medicinal chemistry procedure for the conversion of **9** to the API involved a deprotection step using TFA followed by mesylate salt formation. This procedure was developed using the alanine analogue which was at that time considered a potential candidate. While the same deprotection conditions worked well on the glycine analogue, isolation of the freebase **10** was not possible due to poor solubility in organic solvents. Attempted neutralization of the trifluoroacetate salt followed by an extractive workup using various organic solvents led to a gummy mixture. Meanwhile, a salt selection study of the freebase that was conducted led to the identification of a crystalline monomesylate salt as the preferred salt form for further development (Table 2). We wanted to use methanesulfonic acid (MSA) in a one-pot reaction both to remove the Boc group and to generate the mesylate salt as a crystalline solid in the preferred physical form.[15]

On the basis of small-scale crystallization studies on the mesylate salt formation from the freebase in several solvents, only acetic acid gave a consistent freebase/acid 1:1 ratio . All other solvents examined[16] gave ratios >1.17:1. We established that complete removal of the Boc group required 1.2 mol equiv of MSA[17] at 60 °C, using glacial acetic acid as the solvent. As the reaction mixture was cooled, the mesylate salt slowly crystallized out. To maximize the mass recovery, methyl *tert*-butyl ether (MTBE) was used as an antisolvent which was added prior to cooling. Decomposition of MTBE under these

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Table 2. Selected physical properties of the freebase and the mesylate salt

physical property	freebase	mesylate salt
aqueous solubility	insoluble	1.06 mg/mL
DSC melting endotherm	230 °C	299 °C
moisture sorption	forms a hemihydrate at 20−80% RH forms a monohydrate at 90% RH	2.6% uptake at 90% RH
XRPD	crystalline	crystalline (Form A)

conditions was not an issue as methanol was not detected in the residual solvent analysis. Higher amounts of MSA gave a higher acid/freebase ratio. The optimized process consistently afforded the desired form of the mesylate salt. The isolated API typically contained 1.3−2.0% (w/w) residual acetic acid which was found to be acceptable on the basis of its generally regarded as safe (GRAS) status.[17,18] The optimized deprotection and mesylate salt formation process was implemented at 3.3 kg scale to afford the API in 80% yield and with >99% purity with no detectable impurity arising from the N2 substitution byproduct in the Ullmann coupling reaction. The impurity derived from over-acylation was observed at 0.22%. No other impurities of significance were detected, rendering an effective process (see Scheme 5).

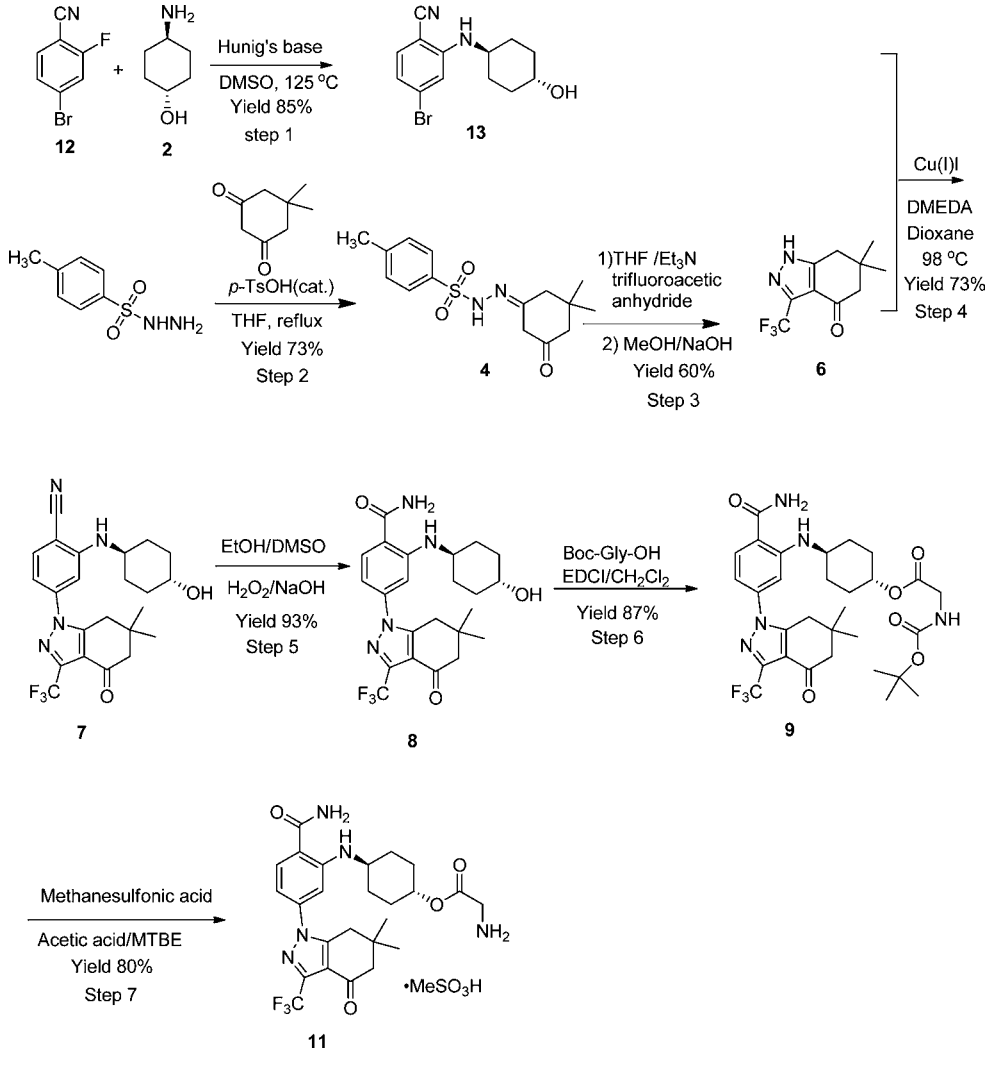
CONCLUSIONS

An improved process for the production of **11** was developed and the process successfully demonstrated under cGMP conditions. The use of 2-fluoro-4-bromobenzonitrile as the starting material selectively afforded the desired alternative key intermediate **13** and significantly improved the overall yield of the process. A copper-mediated Ullmann-type coupling to install the core structure and a one-pot deprotection and salt formation in acetic acid were the keys of the process. The identification and control of impurities at each stage helped establish and demonstrate the robustness of the process to consistently afford the API with >99.5% purity.

EXPERIMENTAL SECTION

The mass spectra were obtained on a Finnigan LCQ-DUO spectrometer using electrospray ionization in the positive mode. HPLC was collected on either a Varian system or a Waters Symmetry system. Nonvalidated HPLC method details (used in developmental runs) and validated API release method

Scheme 5. Improved synthesis of the API



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details are provided in the Supporting Information (% conversion implies integrating only the starting material and product; % purity implies area % of the concerned peak and all other relevant peaks integrated unless otherwise stated).

4-Bromo-2-((*trans*-4-hydroxycyclohexyl)amino)-benzonitrile (13). Bromo-2-fluorobenzonitrile (1.10 kg, 5.50 mol) and *trans*-4-aminocyclohexanol (0.70 kg, 6.05 mol) were taken in DMSO (6.6 L) and treated with diisopropylethylamine (0.71 kg, 5.50 mol). The mixture was heated to 125 °C over 4.5 h and stirred for 5 h (<1% **12** by HPLC). The mixture was cooled to ambient temperature over 3 h, diluted with water (16.5 L), and extracted with ethyl acetate (3 × 4.4 L). The combined organic layers were washed with brine (3 × 3.3 L) and concentrated under vacuum (30 °C) to an end volume of 3.6 L. The slurry was heated to 50 °C over 50 min, and heptanes (6.6 L) was added over 1 h. The mixture was cooled to ambient temperature over 3 h, filtered, and washed with heptanes (2.5 L). The solids were dried under vacuum at ambient temperature for 29 h to afford **13** (1.41 kg, 85% yield) as a pale-yellow solid. ^{1}H NMR (300 MHz, DMSO): δ 1.29−1.40 (m, 4H), 1.81−1.87 (m, 4H), 3.37−3.43 (m, 2H), 4.58 (d, 1H, J = 4.2 Hz), 5.78 (d, 1H, J = 9 Hz), 6.75−6.79 (m, 1H), 7.0 (d, 1H, J = 2 Hz), 7.37 (d, 1H, J = 9 Hz). ^{13}C NMR (75 MHz, DMSO): δ 29.74, 33.68, 50.30, 68.19, 93.54, 114.38, 117.52, 118.49, 128.57, 134.96.

***N′*-(3,3-Dimethyl-5-oxocyclohexylidene)-4-methyl-benzenesulfonohydrazide (4).** Toluenesulfonohydrazide (7.5 kg, 40.27 mol), 5,5-dimethyl-1,3-cyclohexanedione (6.27 kg, 40.27 mol), and *p*-toluenesulfonic acid monohydrate (0.07 kg, 0.40 mol) in tetrahydrofuran (202 L) were heated to 65 °C over 2 h and stirred for 19 h (99.8% **4** by HPLC). The mixture was cooled to 25 °C over 2 h, MTBE (75 L) was added and stirred for 80 min. The slurry was filtered, washed with MTBE (75 L), and dried in a vacuum oven at 25 °C to give **4** (9.94 kg, 80% yield) as a white solid. ^{1}H NMR (300 MHz, DMSO): δ 0.9 (s, 6H), 1.92 (s, 2H), 2.07 (s, 2H), 2.38 (s, 3H), 5.08 (s, 1H), 7.40 (d, 2H, J = 8.1 Hz), 7.69 (d, 1H, J = 8.4 Hz), 8.67 (s, 1H), 9.77 (s, 1H). ^{13}C NMR (75 MHz, DMSO): δ 21.38, 27.18, 28.11, 32.69, 50.64, 96.95, 127.89, 130.0, 136.05, 143.97, 195.38.

6,6-Dimethyl-3-(trifluoromethyl)-6,7-dihydro-1*H*-indazol-4(5*H*)-one (6). TFAA (8.68 kg, 41.3 mol) was added over 15 min to a stirring mixture of **4** (6.37 kg, 20.65 mol) in tetrahydrofuran (96 L). The mixture was heated to 35 °C, and triethylamine (10.45 kg, 103 mol) was added over 30 min and stirred for 2 h (**4** undetected by HPLC). The mixture was cooled to 25 °C over 3 h, and NaOH solution (1 M in methanol, 25 L) was added in one portion and stirred for 19 h (0.5% **5** by HPLC). The mixture was quenched with 20% aqueous ammonium chloride (64 L) and extracted with ethyl acetate (2 × 45 L). The combined extracts were filtered through a pad of silica gel and concentrated under vacuum (30−35 °C) to an end volume of 15−16 L. The mixture was heated to 40 °C and heptanes (13 L) added. The mixture was cooled to 20 °C over 3 h, filtered, washed with ethyl acetate/heptanes (1:1, 30 L), and dried in a vacuum oven at 25 °C to give **6** (7.04 kg, 60% yield, 99.8% purity by HPLC) as an orange solid. ^{1}H NMR (300 MHz, DMSO): δ 1.03 (s, 6H), 2.36 (s, 2H), 2.80 (s, 2H), 13.90 (bs, 1H). ^{13}C NMR (75 MHz, DMSO): δ 25.47, 26.25, 32.45, 33.96, 50.83, 112.26, 121.43 (q, J = 270 Hz), 136.36, 138.86, 150.74, 188.72. ^{19}F (282 MHz, DMSO): δ −61.96.

4-(6,6-Dimethyl-4-oxo-3-(trifluoromethyl)-4,5,6,7-tetrahydro-1*H*-indazol-1-yl)-2-((*trans*-4-hydroxycyclohexyl)amino)benzonitrile (7). Compound **13** (3.50 kg, 11.86 mol), **6** (2.48 kg, 10.68 mol), and potassium carbonate (3.45 kg, 24.96 mol) in anhydrous 1,4-dioxane (28 L) were sparged with N_2(g) for 30 min. A degassed solution of cuprous iodide (0.45 kg, 2.37 mol in 7 L of dioxane) and DMEDA (0.56 L, 5.15 mol in 1.41 L of dioxane) were added. The mixture was heated to 98 °C over 2 h and stirred for 65 h (<5% of **6** by HPLC). The mixture was cooled to ≤30 °C and treated with concentrated NH_4OH solution (4.74 L), DI water (11 L), and saturated NH_4Cl (15.8 L). The organic phase was separated and concentrated under vacuum (40 °C) to an end volume of 11−12 L. The mixture was heated to 60 ± 5 °C, and *n*-heptane (13.2 L) was added over 2 h at ≥50 °C. The slurry was cooled to 20 °C over 66 h, filtered, washed with ethyl acetate/*n*-heptane (1:2, 21 L). The wet cake taken in ethyl acetate (21 L) was heated to 60 ± 5 °C over 25 min and stirred for 3 h. *n*-Heptane (21 L) was added over 2 h at ≥50 °C, cooled to 20 °C over 18 h, and filtered. The cake was washed with ethyl acetate/*n*-heptane (1:2, 13.5 L) and dried in a vacuum oven at 45 ± 5 °C for 22 h to give **7** (3.49 kg, 73% yield, 99.6% purity by HPLC analysis) as a white powder. ^{1}H NMR (300 MHz, DMSO): δ 1.05 (s, 6H), 1.28−1.45 (m, 4H), 1.83−1.95 (m, 4H), 2.46 (s, 2H), 3.01 (s, 2H), 3.40−3.47 (m, 2H), 3.57 (s, 3H), 4.59 (d, 1H, J = 3 Hz), 6.07 (d, 1H, J = 6 Hz), 6.87−6.90 (m, 1H), 7.06 (d, 1H, J = 1.5 Hz), 7.67 (d, 1H, J = 9 Hz). ^{13}C (75 MHz, DMSO): δ 27.46, 29.76, 33.77, 35.15, 35.96, 50.73, 51.63, 66.33, 68.25, 94.73, 107.60, 111.17, 115.57, 117.38, 123.3 (q, J = 235 Hz) 135.17, 142.00, 150.41, 152.17, 190.09. ^{19}F (282 MHz, DMSO): δ −62.27. Water content <0.1% (w/w by Karl Fisher analysis); residual copper =14 ppm (ICP analysis).

4-(6,6-Dimethyl-4-oxo-3-(trifluoromethyl)-4,5,6,7-tetrahydro-1*H*-indazol-1-yl)-2-((*trans*-4-hydroxycyclohexyl)amino)benzamide (8). Sodium hydroxide solution (1 N, 0.65 L) was added over 10 min to a stirring mixture of **7** (3.25 kg, 7.28 mol) in ethanol (26 L) and DMSO (6.5 L) at 10 °C. Aqueous hydrogen peroxide (30 wt %, 0.65 L) was added over 2 h at 15−25 °C (**CAUTION**: hydrogen peroxide can be hazardous. Use appropriate precautions!) and stirred for 21 h (**7** undetected by HPLC). The batch was cooled to ≤10 °C over 2 h and treated with cold (5 °C) sodium thiosulfate solution (10 wt %, 52 L) and DI water (37 L). The solids were filtered, washed with deionized water (42 L), and dried in a vacuum oven at 50 ± 5 °C to give **8** (3.16 kg, 93% yield, 99.6% purity by HPLC) as a white solid. ^{1}H NMR (300 MHz, DMSO): δ 1.04 (s, 6H), 1.17−1.35 (m, 4H), 1.80−1.84 (m, 2H), 1.98−2.01 (m, 2H), 2.45 (s, 2H), 2.99 (s, 2H), 3.46−3.49 (m, 2H), 4.58 (d, 1H, J = 6 Hz), 6.70−6.73 (dd, 1H, J = 8.4 and 1.8 Hz), 6.87 (d, 1H, J = 1.5 Hz), 7.32 (bs, 1H), 7.78 (d, 1H, J = 8.4 Hz), 7.98 (bs, 1H), 8.38 (d, 1H, J = 9 Hz). ^{13}C (75 MHz, DMSO): δ 27.55, 30.07, 33.38, 35.18, 36.09, 49.51, 51.72, 68.02, 106.88, 108.93, 113.99, 115.38, 120.58 (q, J = 234 Hz) 130.72, 140.68, 149.66, 151.97, 170.83, 190.22. ^{19}F (282 MHz, DMSO): δ −62.10. Water content <0.1% (w/w by Karl Fisher analysis); residual copper = 3 ppm (ICP analysis).

(*trans*-4-((2-Carbamoyl-5-(6,6-dimethyl-4-oxo-3-(trifluoromethyl)-4,5,6,7-tetrahydro-1*H*-indazol-1-yl)-phenyl)amino)cyclohexyl)-2-((*tert*-butoxycarbonyl)-amino)acetate (9). Compound **8** (3.05 kg, 6.57 mol), Boc-glycine (2.32 kg, 13.25 mol), EDCI (2.54 kg, 13.23 mol), and

DMAP (0.08 kg, 0.68 mol) were taken together in methylene chloride (61 L) and stirred for 21 h at 18−23 °C (0.8% **8** by HPLC). Purified water (61 L) was added, and phases were separated. The aqueous phase was back extracted with methylene chloride (61 L) and the combined organic layer washed successively with saturated $NaHCO_3$ solution (61 L), 1 N HCl (61 L), and saturated NaCl solution (61 L). The organic phase was concentrated under vacuum (35 °C) to an end volume of 10−11 L and swapped with ethyl acetate. The mixture was heated to 50 ± 5 °C over 15 min, and *n*-heptane (24 L) was added over 1 h and then cooled to 20 °C over 22 h. The mixture was filtered, washed with ethyl acetate/*n*-heptane (1:2, 11 L), dried in a vacuum oven at 40 ± 5 °C to give crude **9** (4.14 g, 101% yield). A second batch repeated on similar scale was combined and purified together. Crude **9** (8.24 kg, 13.26 mol) in ethyl acetate (41.5 L) was heated to 75 ± 5 °C over 2 h, stirred for 6 h, and cooled to 50 ± 5 °C. The mixture was filtered, washed with ethyl acetate (8.3 L), and dried in a vacuum oven at 40 ± 5 °C to give **9** (7.20 kg, 87% yield, 99.5% purity by HPLC with 0.16% of impurity 2) as a white solid. ^{1}H NMR (300 MHz, DMSO): δ 1.04 (s, 6H), 1.38−1.45 (m, 2H), 1.42 (s, 9H), 1.53−1.60 (m, 2H), 1.90−1.93 (m, 2H), 1.99−2.06 (m, 2H), 2.45 (s, 2H), 2.99 (s, 2H), 3.35 (s, 2H), 3.47−3.49 (m, 1H), 3.64−3.66 (d, 2H, J = 6 Hz), 4.73−4.76 (m, 1H), 6.72−6.76 (dd, 1H, J = 8.4 and 1.8 Hz), 6.91 (d, 1H), 7.18−7.22 (t, 1H, J = 6.1 Hz), 7.35 (s, 1H), 7.80 (d, 1H, J = 9 Hz), 8.02 (bs, 1H), 8.4 (d, 1H, J = 7.5 Hz). ^{13}C (75 MHz, DMSO): δ 28.58, 28.98, 29.23, 30.13, 30.30, 36.23, 37.10, 43.28, 49.77, 52.79, 60.81, 73.13, 79.24, 101.93, 110.17, 115.14, 116.43, 123.49 (q, J = 235 Hz), 131.81, 139.22, 141.73, 150.58, 153.03, 156.93, 170.88, 171.84, 191.23; ^{19}F (282 MHz, DMSO): δ −62.12. Copper: <1 ppm (ICP analysis).

(*trans*-4-((2-Carbamoyl-5-(6,6-dimethyl-4-oxo-3-(trifluoromethyl)-4,5,6,7-tetrahydro-1*H*-indazol-1-yl)-phenyl)amino)cyclohexyl)-2-aminoacetate Methanesulfonate (11). Compound **9** (3.30 kg, 5.31 mol) in glacial acetic acid (23 L) was stirred until complete dissolution and filtered through a 10 μm inline filter. Methanesulfonic acid (0.62 kg, 6.43 mol) was added over 15 min, heated to 65 ± 5 °C over 40 min and stirred for 3 h (**9** undetected by HPLC). The mixture was cooled to 50 ± 5 °C over 55 min, and MTBE (16 L) was added. The batch was cooled to 20−25 °C over 17 h, filtered, and washed with acetic acid (1.65 L) and then MTBE (9.7 L). The solids were dried in a vacuum oven at 55 °C to give **11** (2.625 kg, 80% yield, 99.5% purity by HPLC) as a white solid. ^{1}H NMR (300 MHz): δ 1.04 (s, 6H), 1.38−1.45 (m, 2H), 1.55−1.66 (m, 2H), 1.91−2.08 (m, 4H), 2.34 (s, 3H), 2.45 (s, 2H), 2.99 (s, 2H), 3.36 (s, 1H), 3.39−3.52 (m, 1H), 3.83 (s, 2H), 4.83−4.89 (m, 1 H), 6.72−6.76 (dd, 1H, J = 8.3 and 1.8 Hz), 6.92 (d, 1H, J = 1.75 Hz), 7.36 (bs, 1H), 7.8 (d, 1H, J = 8.4 Hz), 8.02 (m, 1H), 8.22 (bs, 2H), 8.4 (d, 1H, J = 7.5 Hz). ^{13}C (75 MHz, DMSO): δ 27.46, 28.88, 29.07, 35.10, 35.95, 48.51, 51.66, 73.49, 106.83, 109.11, 114.05, 115.30, 124.1 (q, J = 234 Hz), 130.69, 137.56, 138.08, 138.60, 139.11, 140.60, 149.46, 151.93, 167.09, 170.71, 171.90, 190.11. ^{19}F (282 MHz, DMSO): δ −62.92. Copper: <1 ppm (ICP analysis); DSC melting endotherm: 291 °C; residual acetic acid: 1.4% (w/w); residual MTBE: 460 ppm.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

NMR and HPLC data for all intermediates on laboratory scale (1 kg) process demonstration batch. NMR and HPLC data of the API from the cGMP batch using the release HPLC method including details of the release HPLC method. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) (a) Maloney, A.; Workman, P. *Expert Opin. Biol. Ther.* **2002**, *2*, 3−24. (b) Dymock, B. W.; Drysdale, M. J.; McDonald, E.; Workman, P. *Expert Opin. Ther. Pat.* **2004**, *14*, 837−847. (c) Isaacs, J. S.; Wu, X.; Neckers, L. *Cancer Cell* **2003**, *3*, 213−217.

(2) Huang, K. H.; Eaves, J.; Hanson, G.; Veal, J.; Barta, T.; Geng, L.; Hinkley, L. Tetrahydroindolone and tertrahydroindazolone derivatives. WO/2006/091963, 2006.

(3) Overall yield in this main sequence was 40% compared to 6% using the alternative palladium chemistry (details of yields discussed in ref 8).

(4) Klapars, A.; Antilla, J. C.; Huang, X.; Buchwald, S. L. *J. Am. Chem. Soc.* **2001**, *123*, 7727.

(5) Thermal analysis was done using Thermal Screening Unit from HEL.

(6) The optimal reaction temperature was found to be 125 °C, and at lower temperatures the reaction significantly slowed down.

(7) Hiegel, A. G.; Burk, P. *J. Org. Chem.* **1973**, *38*, 3637−3638.

(8) Huang, K. H.; et al. *J. Med. Chem.* **2009**, *52*, 4288−4305.

(9) A small amount of the impurity was isolated and characterized by ^{1}H NMR and ^{13}C NMR.

(10) Reference 8 describes unsuccessful attempts to isolate the acylated triketone. The trifluoroacyl intermediate in the absence of sufficient energy to cyclize undergoes decomposition.

(11) Antilla, J. C.; Baskin, J. M.; Barder, T. E.; Buchwald, S. L. *J. Org. Chem.* **2004**, *69*, 5578−5587.

(12) Ma, D.; Cai, Q.; Zhang, H. *Org. Lett.* **2003**, *5*, 2453−2455.

(13) Hasegawa, T.; et al. *Org. Process Res. Dev.* **2005**, *9*, 774−781.

(14) Structure of this impurity was proposed by MS−MS analysis and also by characterizing the corresponding derivative in the API.

(15) Green, T. W. *Protecting Groups in Organic Synthesis*; John Wiley & Sons: New York, 1999. (b) Kochienski, P. *Protecting Groups*, 3rd ed.; Thieme: New York, 2004; p 461.

(16) Solvents examined were acetonitrile, dioxane, ethyl acetate, isopropyl acetate, THF, DMF, NMP, DMA, and DMSO.

(17) (a) Rolf, J. Synthesis of cyclohexylglycine benzyl esters. DE 19920907, 2000. (b) Yamashita, Y.; Moroshima, T.; Ueda, Y. Jpn. Kokai Tokkyo Koho JP 2003183230, 2003.

(18) Stahl, P. H., Vermuth, C. G., Eds. *Handbook of Pharmaceutical Salts: Properties, Selection and Use*; Wiley-VCH: Weinheim; 2002.