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Development of an Effective Palladium Removal Process for VEGF Oncology Candidate AG13736 and a Simple, Efficient Screening Technique for Scavenger Reagent Identification

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Abstract:

AG13736 (Axitinib), an inhibitor of vascular endothelial growth factor (VEGF) under investigation as an oncology drug, is currently manufactured via a three-step process that utilizes two palladium-mediated cross-couplings. Historically, removal of residual heavy metals from the active pharmaceutical ingredient has been a persistent issue. The development of a much improved process for palladium removal and a useful screening technique developed to rapidly identify the most efficient reagents for this purpose are outlined. The performance of the new endgame process in pilot-plant scale-up is also discussed.

1. Introduction

Vascular endothelial growth factor (VEGF) is the most common target of a number of anti-angiogenesis agents under development as anticancer drugs. This promising class of drugs act to prevent the growth and proliferation of cancer cells via interruption of tumor angiogenesis (formation of vascular supply tissue), and there are currently about 25 such agents under study in clinical trials.[1] Axitinib (**1**), a VEGF inhibitor in Pfizer's oncology development program, is currently manufactured via a three-step process from readily available starting materials (Scheme 1). This synthetic route represents an improvement over the first-generation manufacturing route that utilized *N*-tetrahydropyranyl-protection (THP) of the indazole core and totaled seven steps (Scheme 2).[2] While each of these syntheses employ a different connective approach, both syntheses feature the use of palladium metal in two manufacturing steps, and both have shared one common issue: the removal of residual palladium. Whereas palladium removal has been carried out prior to the last synthetic step in the first-generation synthesis, an efficient palladium removal process is particularly critical for the newer version since Pd is used in the final reaction step forming the active pharmaceutical ingredient (API or "drug substance"). This paper will highlight the recent advances in the development of an efficient process for removal of residual palladium from Axitinib drug substance material.

2. Background

The wealth of important carbon−carbon (and carbon−heteroatom) bond-forming reactions available through palladium-mediated processes renders chemistry with this metal an indispensable part of manufacturing in the pharmaceutical and fine chemicals industries. One potential drawback to the use of palladium or other heavy metals in manufacturing processes is that removal of residual metal from the API or intermediates is often problematic. The emergence of Pd-mediated chemistry in modern pharmaceutical manufacturing has been attended by the development of specialized techniques to sequester and remove unwanted metal contaminants.[3] Among the more commonly used materials for palladium removal are metal-coordinating agents covalently bound to inorganic or polymeric supports, with a number of these available from commercial suppliers. The commercially available reagents are often very effective and broadly applicable, but they are usually quite expensive and in the case of agents bound to inorganic supports, the palladium metal may not be easily recoverable (e.g., via incineration). Other cheaper options that are effective in some cases include common adsorbents such as activated carbon or Fuller's earth, or soluble (unsupported) scavengers.

Axitinib and intermediates thereto have exhibited a tendency to retain palladium, a fact that likely is due to the heteroatom-rich structure of the core molecule that may enable formation of a relatively stable complex with the metal. As a result, separation of the compound(s) from residual palladium has been a challenging task. For the first-generation synthesis, removal was carried out just prior to formation of the API in a subsequent final deprotection step (Scheme 2). Since intermediate **13** is soluble in a range of organic solvents, early work focused on the use of *insoluble* scavenger materials to remove metal from solutions of the crude compound. The most effective treatments were thiol-containing reagents on solid supports from commercially available sources. One improvement made was the

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(1) Ferrara, N. *Oncologist* **2004**, *9* (suppl. 1), 2.

(2) Steps for the purpose of this comparison include only bond-forming reactions, regardless of whether steps are combined in a single-pot operation. Both syntheses include endgame processing steps to remove residual heavy metals, achieve chemical purification and effect control of the final polymorphic form. The purpose of this paper is to discuss work toward an effective palladium removal process; discussion of the preceding chemistry and polymorph control steps are beyond the scope of this manuscript.

(3) (a) Garrett, C. E.; Prasad, K. *Adv. Synth. Catal.* **2004**, *346*, 889. (b) Bien, J. T.; Lane, G. C.; Oberholzer, M. R. *Top. Organomet. Chem.* **2004**, *6*, 263.

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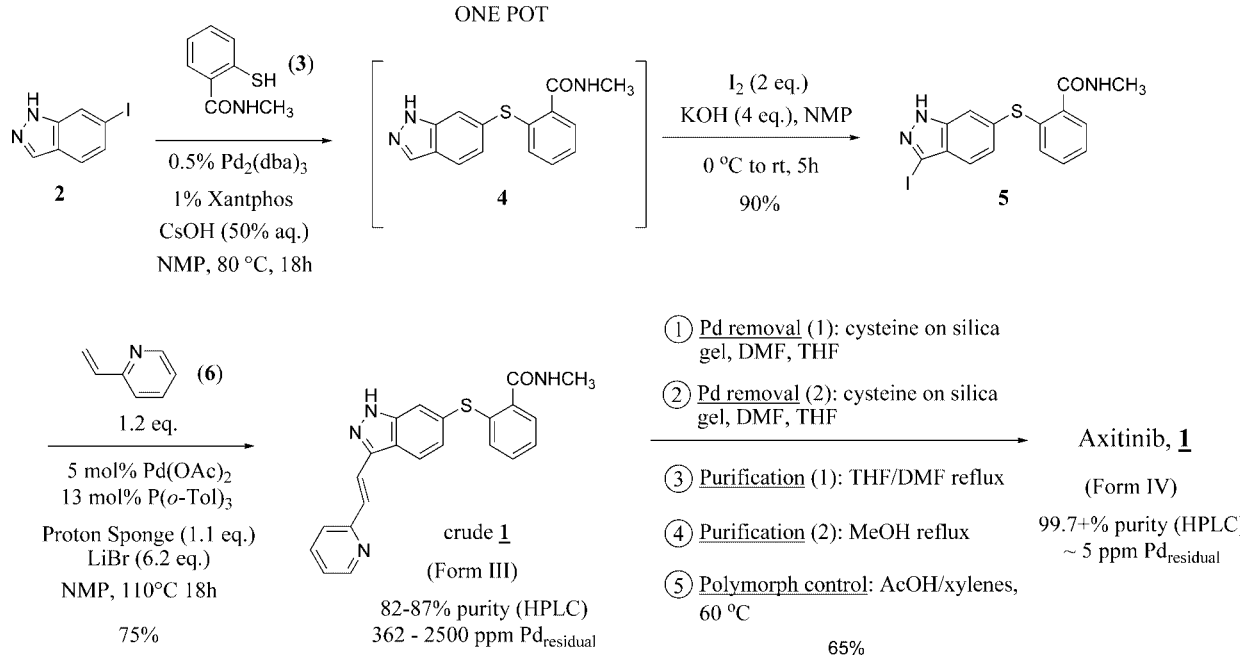
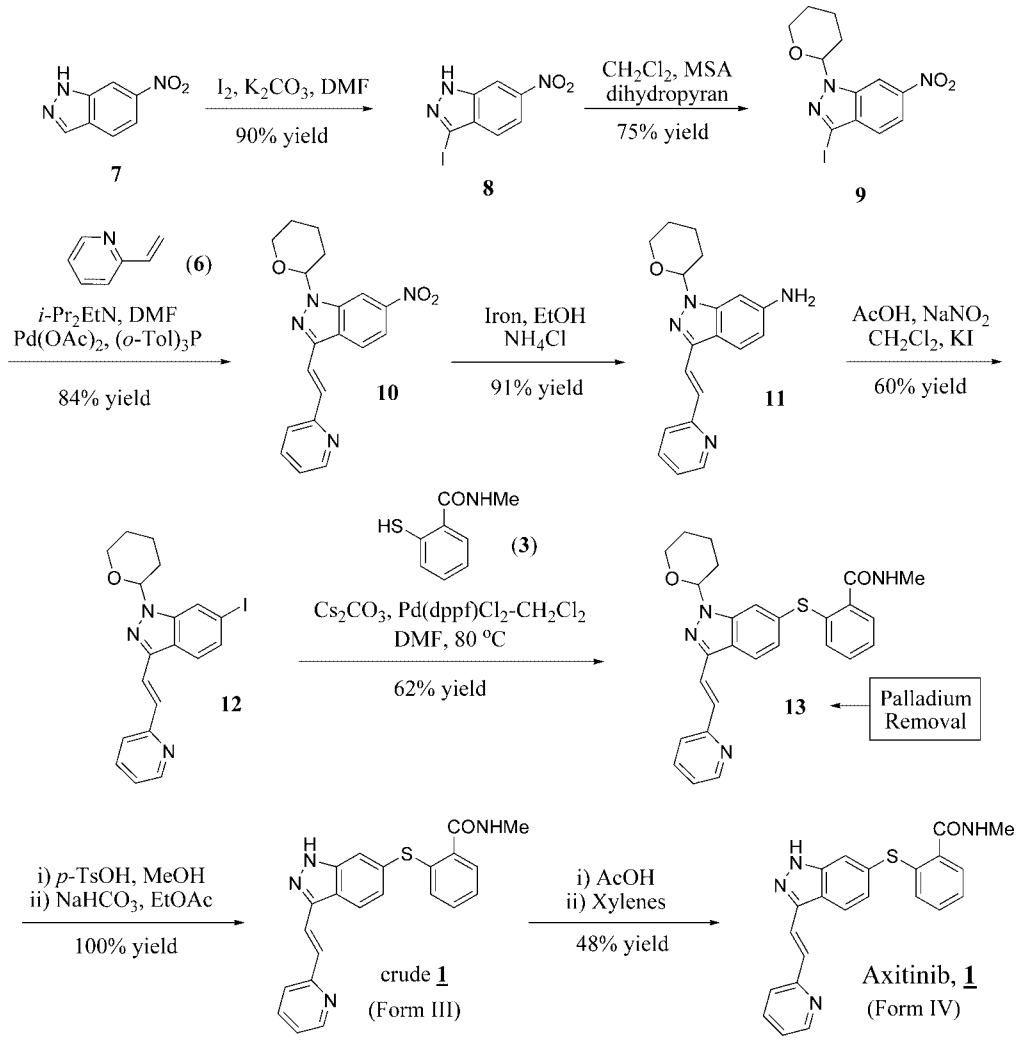
Scheme 1. **New phase II manufacturing route**



Scheme 2. **First-generation manufacturing route**



use of 10 wt %/wt cysteine *adsorbed* on silica gel, a cheaper alternative to the commercial reagents which can also be filtered away after use. Although a considerable amount (2 × 5 wt equiv) was needed to reduce Pd to below 20 ppm, this treatment

was an effective remedy that was used several times in large-scale manufacturing campaigns.

When a shorter and more efficient synthesis was identified, a first-generation process for removal of palladium from the API was patterned after the original using the 10% cysteine/silica gel reagent once again. Due to the markedly different physical properties of **AG13736** versus those of intermediate **13**, the cysteine treatment was not as effective from a practical point-of-view, and the resulting process was cumbersome and performed poorly when run at kilogram scale. The first-generation palladium removal process is depicted in Scheme 1. Following the Heck reaction, crude **AG13736** (85% HPLC purity) was dissolved in 1:1 tetrahydrofuran/*N*,*N*-dimethylacetamide (1:1 THF−DMAc) and treated with 5 wt equiv of 10% cysteine on silica gel; after filtration of the solids, the THF solvent was stripped and product was recovered from the DMAc filtrate via addition of a large volume of water. The aforementioned process was then repeated to obtain product containing less than 20 ppm residual palladium (87% HPLC purity). Next, two separate heated reslurries were carried out (methanol (MeOH) and 9:1 THF−DMAc) to achieve the required overall purity specification.

There were a number of obvious drawbacks to the use of the cysteine treatment in the context of the new synthesis. First, complete removal of palladium required two sequential treatments of *5 wt equiv* of the reagent, separated by workup and isolation of product. Second, the lack of solubility of **AG13736** in midrange to lower polarity solvents required that polar solvents such as *N*-methylpyrrolidone (NMP) or *N*,*N*-dimethylacetamide be used. As a result, some leaching of the cysteine from the silica gel support into the polar media was observed, and the filterability of the spent silica reagent was very poor. Third, the overall removal process was very cumbersome in terms of the number of operations, and only palladium metal was removed without any purging of chemical impurities, which required the latter to be dealt with in additional purification operations. While we had originally planned to enable the palladium removal process outlined above to meet our short-term manufacturing needs, it soon became clear that this process might fail completely in the pilot plant even in a spot-use type context. Rapid development of a more robust and economical process for palladium removal was therefore essential to meet delivery expectations for phase II clinical studies.

3. Results and Discussion

3.1. Matching API Physical Properties with Pd Removal Technique (Initial Work). The API-forming, final reaction in the second-generation synthesis outlined in Scheme 1 is a Heck reaction between iodoindazole intermediate **5** and 2-vinylpyridine (**6**) employing NMP as solvent. The reaction is performed under conventional conditions but using a base that is less conventional in the context of this reaction, proton sponge.[4] Isolation of product from the crude reaction mixture was somewhat problematic and a number of options were explored before settling on a somewhat cumbersome, but reasonably consistent extractive workup for the phase II manufacturing campaign. This isolation method involves partitioning of the crude reaction mixture between dilute hydrochloric acid (HCl) and methyl isobutyl ketone (MIBK), prior to precipitation of the crude product from the aqueous extract via addition of concentrated NH_4OH in the presence of added toluene to dissolve coprecipitated proton sponge. Filtration affords the crude Heck product as a yellow solid typically containing 1000−2500 ppm of residual Pd metal. While alternative, precipitative isolation options produced material of varied residual Pd content (generally higher than 2000 ppm), extensive endgame processing was required to remove palladium and meet the required purity specification whatever the workup.

Our evaluation of the previously established Pd-removal process revealed that this approach did not sufficiently take the physical properties of the substrate into account. Specifically, an *insoluble* supported reagent seemed ill-suited for removal of Pd metal from a compound that is itself quite *insoluble*. In redesigning an endgame process, it made more sense to allow the crude **AG13736** to remain as the solid component and for metal contaminants to be removed to a liquid supernatant containing soluble scavengers by either of two means: recrystallization or reslurrying ("leaching" of palladium into the liquid phase). Further, this approach could allow for palladium removal and chemical purification to be combined in a single operation, since impurities would likely be retained in the supernatant to at least some extent.

Our first sets of experiments were based around the concept of "leaching" the metal contaminants into a solvent in which **AG13736** was not particularly soluble (e.g., reslurries). A solubility profile comparison was conducted to identify the most favorable solvents for this purpose. A number of milligram-scale reslurries were carried out in a range of process-friendly solvents and solvent mixtures, and the resulting supernatants were analyzed. This was a very rapid and effective means to generate lead solvents for a reslurry process, since HPLC analysis of the supernatant provides not only solubility data for the main component (and thus qualitative product recovery information) but also relative product/impurity solubility data (and thus purge quality information). Although palladium removal could also conceivably be quantified via appropriate analyses of the supernatant, in our case inspection of the color of the supernatant provided a fast and convenient means to qualitatively assess the amount of palladium removed to the solution phase (verified with a few test analyses). The best solvent mixtures for reslurrying **AG13736** based on a combination of potential recovery, impurity purging, and palladium removal potential were 4:1 THF−MeOH, 4:1 methyl ethyl ketone−water, and 9:1 THF−water.

Initial results with solvents alone indicated that a surprisingly large amount of palladium could be removed from the solid substrate simply via heating a suspension under stirring (Table 1). However, in no case was the extent of palladium removal near to the required benchmark of <20 ppm. Attempts to effect further removal of palladium via sequential reslurrying or "continuous extraction" techniques were attended by a general leveling off of the amount of palladium removed. There was some evidence (by Raman spectroscopy) in the methanol examples that continued reslurrying in this solvent was ac-

(4) A separate manuscript detailing the development of the chemistry for the synthesis depicted in Scheme 1 is planned.

Table 1. **Initial reslurry and crystallization results with various metal ligands**

entry[a]	solvent (10 vol)	scavenger[b]	crystallized?	reslurry?	$Pd_{residual}$[c]	recovery (%)
1	MeOH (first reslurry)	−		x	2,085 ppm (18%)	N/A
2	MeOH (2nd reslurry)	−		x	1,363 ppm (46%)	77
3	MeOH (3rd reslurry)	−		x	1,088 ppm (57%)	N/A
4	9:1 MeOH−DMF	−		x	1,117 ppm (56%)	70
5	4:1 MeOH−AcOH	−		x	481 ppm (81%)	75[d]
6	9:1 THF−DMAc	−		x	589 ppm (77%)	85
7	9:1 MeOH−DMF	EDTA		x	359 ppm (86%)	N/A
8	9:1 MeOH−DMAc	EDTA		x	222 ppm (91%)	N/A
9	MeOH	En		x	1,143 ppm (55%)	85
10	4:1 THF−MeOH	En		x	92 ppm (96%)	96
11	4:1 THF−MeOH	−		x	845 ppm (67%)	79
12	4:1 MEK−water	En		x	2,278 ppm (10%)	95
13	4:1 MEK−water	−		x	2,957 ppm (0%)	100
14	NMP (MeOH)	En	x		483 ppm (81%)	89
15	NMP (MeOH)	En[e]	x		399 ppm (84%)	N/A
16	NMP (MeOH)	En[f]	x		110 ppm (95%)	N/A
17	4:1 THF−MeOH	1,2-Pn		x	368 ppm (87%)	71[g]
18	4:1 MEK−water	1,2-Pn		x	1859 ppm (27%)	N/A
19	EtOH	1,2-Pn		x	1441 ppm (43%)	N/A
20	1:1 MeOH−NMP	1,2-Pn		x	612 ppm (76%)	N/A
21	NMP (MeOH)	1,2-Pn	x		165 ppm (93%)	72
22	NMP (MeOH)	1,2-Pn	x		43 ppm (98%)	65[g]
23	NMP (MeOH)	1,2-Pn[e]	x		221 ppm (94%)	N/A
24	NMP (MeOH)	1,2-Pn[f]	x		213 ppm (94%)	N/A
25	NMP (MeOH)	−	x		1179 ppm (53%)[h]	N/A
26	NMP (MeOH)	−[e]	x		864 ppm (65%)	N/A
27	9:1 MeOH−DMF	NAC		x	N/A[i]	N/A
28	9:1 MeOH−DMAc	NAC		x	2,223 (12%)	N/A
29	2:1 THF−1.2 N $HCl_{(aq)}$	NAC		x	517 ppm (79%)	N/A
30	9:1 MeOH−DMF	TSA		x	847 ppm (67%)	N/A
31	9:1 MeOH−DMAc	TSA		x	997 ppm (61%)	N/A
32	4:1 THF−MeOH	Dien		x	562 ppm (78%)	49
33	NMP (MeOH)	Dien	x		196 ppm (92%)	N/A
34	4:1 THF−MeOH	Tren		x	515 ppm (80%)	51
35	NMP (MeOH)	Tren	x		406 ppm (84%)	N/A
36	NMP (MeOH)	Trien	x		290 ppm (89%)	N/A

[a] All experiments used **AG13736** material initially containing 2531 ppm of residual Pd. [b] Standard conditions: 250−500 mg **AG13736**, 1 wt equiv of scavenger, 10 vol of solvent, stirred at reflux for 3.5−19 h except as otherwise noted. Experiments in NMP were carried out at RT and employed an additional granulation period (1−3 days) after addition of MeOH antisolvent (20 vol) except as otherwise noted. Scavenger abbreviations: *EDTA* = ethylenediaminetetraacetic acid; *NAC* = *N*-acetyl-cysteine; *TSA* = thiosalicylic acid; *En* = 1,2-ethylenediamine; *1,2-Pn* = 1,2-diaminopropane; *Dien* = diethylenetriamine; *Trien* = triethylenetetramine; *Tren* = tris(2-aminoethyl)amine. [c] Number in parentheses represents the percentage of Pd removed based on the starting Pd content. [d] Acetate salt formed. [e] Heated at 100 °C for 5.5 h. [f] Scavenger (En or 1,2-Pn) in higher proportion as"solvent" in the ratio 4:1 scavenger−NMP. [g] Reaction run at 10-g scale using **AG13736** lot containing 2807 ppm residual Pd; product obtained was a white solid, HPLC purity >98%. [h] Preliminary results suggest that crystallization alone from NMP/MeOH can reduce residual Pd content by up to 94% in unusually high-Pd content material (5100 ppm → 315 ppm). [i] Salt with NAC soluble (no ppt).

companied by a polymorph change (to a more stable and presumably less soluble form), which could in part explain the leveling-off effect observed with regard to residual metal content. This example underscores a potential shortcoming of the reslurry approach to palladium removal since solubility and hence the performance of the removal process, will be affected by any physical-form issues that might arise either before the treatment or in the course of the treatment process. For this reason, attention going forward was focused on the recrystallization strategy, since the substrate material fully dissolves and thus polymorph issues are not as much of a concern. Nevertheless, combining the best result from the solvent-screening work (4:1 THF−MeOH) with a number of potential scavengers provided some early indications that the soluble scavenger approach could work for our process and provided the impetus for devising a screen that would identify the best metal-coordinating reagents for our removal process. Early work identified diamine-containing chelating agents such as ethylenediamine and ethylenediaminetetraacetic acid (EDTA) as the most effective as shown in Table 1.

Interestingly, crystallization (NMP/MeOH, acetic acid (AcOH), or AcOH−MeOH) in the absence of metal-coordinating additives was marginally effective for removal of palladium (e.g., entries 25, 26 in Table 1) but not significantly more effective than reslurrying in the absence of such additives (e.g., entries 5, 6 in Table 1). Clearly, it is the presence of an appropriate metal-coordinating additive in the supernatant that makes the difference in the performance of the removal technique (entries 10/11 and 22/25 in Table 1). Possible disruption of the ability of **AG13736** to coordinate with residual palladium through formation of a salt of **AG13736** (acetate, tosylate) as part of the crystallization process did not enhance metal removal (data not shown), nor was treatment with activated carbon effective to any significant and reproducible extent. There are numerous examples found in the literature where final removal of palladium or other metals is accomplished via crystallization of a salt form.[5] While the list of available solvents for **AG13736** crystallization is limited to highly polar types for solubility reasons, one solvent combination that emerged as a very effective option was NMP−MeOH. **AG13736** dissolves in a relatively small volume of NMP and crystallizes upon addition of a reasonable volume of methanol. The resulting supernatant remains very polar and hence well-suited for retention of impurities and, potentially, ligated palladium. Using this solvent

Scheme 3. **Recrystallization and reslurry pilot experiments (10-g scale)**

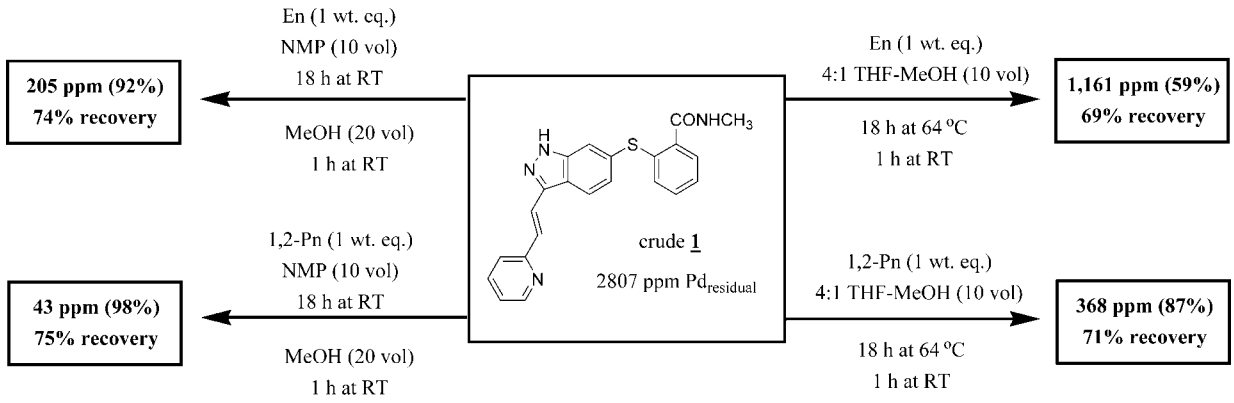


Table 2. **Experiments comparing diamines and diphosphines, alone and in combination**

entry[a]	solvent (10 vol)	scavenger [wt equiv]	$Pd_{residual}$[b]	recovery (%)
1	4:1 THF−MeOH	En [1]	822 ppm (71%)	60
2	4:1 THF−MeOH	DPPE [1]	N.D.	76
3	4:1 THF−MeOH	En [0.5]/DPPE [0.5]	21 ppm (99%)	64
4	4:1 THF−MeOH	En [1]	276 ppm (94%)	64
5	4:1 THF−MeOH	DPPE [1]	212 ppm (95%)	68
6	4:1 THF−MeOH	En [0.5]/DPPE [0.5]	133 ppm (97%)	66
7	NMP (MeOH)	Pn [1]	30 ppm (99%)	79
8	NMP (MeOH)	DPPE [1]	124 ppm (96%)	78
9	NMP (MeOH)	Pn [0.5]/DPPE [0.5]	31 ppm (99%)	78
10	1:1 DMAc−THF	Si-thiol-3 (Silicycle)	148 ppm (95%)	53
11	1:1 DMAc−THF	Si-TAAcOH (Silicycle)	906 ppm (68%)	65
12	1:1 DMAc−THF	Si-thiol-3/Si-TAAcOH	74 ppm (97%)	48

[a] Entries 1−3, 7−12 used **AG13736** material containing 2807 ppm Pd. Entries 4−6 used **AG13736** material containing 4401 ppm Pd. Reaction conditions: heated at 65 °C for 12 h, cooled to RT for 1 h, then filtered (entries 1−6, 10−12); in the case of entries 10−12, product was recovered from the filtrate via addition of water (20 vol). Entries 7−9 were stirred for 12 h at RT, and then MeOH (20 vol) was added; after 18 h, the product was collected by filtration. [b] Numbers in parentheses represent the percentage of Pd removed based on the starting Pd content.

system, a number of dissolved scavengers were tested, and the diamine series of chelating agents were generally found to be most effective. Among these, 1,2-diaminopropane was most effective and offered the best balance between efficiency and cost going forward for development of a viable removal process for the pilot plant. A direct comparison between 1,2-Pn and En in the heated reslurry approach (4:1 THF−MeOH) and NMP crystallization approach on 10-gram scale confirmed that the latter approach with 1,2-Pn was by far the superior lead (Scheme 3). The use of dissolved thiourea or *N*-acetyl-cysteine to sequester palladium during purification by crystallization has been described in a previous issue of OPRD.[6]

3.2. Combinations of Ligands and Scale-Up Results. One issue that was encountered in our initial optimization studies was that significant variability in the performance of a given scavenger was observed with **AG13736** lots that were isolated from the Heck reaction via different methods.[7] It should be noted that within the same lot or between different lots synthesized/isolated in the same manner, there was essentially no variation in the % removal of palladium under the same experimental conditions. It was initially considered that the variability in performance could be linked to variation in the palladium oxidation-state distribution between lots that were isolated in different manners. It was reasoned that this possibility could be tested via treatment of various lots with agents that tend to coordinate with mainly Pd^{2+} (σ-donors, thiols, amines) or Pd^0 (π-acceptors, phosphines, EDTA) alone or in combination. Initial experiments with En and DPPE (1,2-bis(diphenylphosphino)ethane, or DIPHOS) suggested that there may be some modest additive effect to using these reagents in combination (Table 2). This pattern was also observed with the silica-supported reagents Si-TAAcOH (supported EDTA) and Si-thiol (supported ethanethiol) obtained commercially.[8] Although the pattern was far less pronounced using 1,2-Pn as the diamine reagent, the combination of this diamine with DPPE presented, for the very first time, a means to consistently achieve the desired specification for residual Pd (<20 ppm) after a single treatment operation. The addition of a larger excess of the diamine or sequential treatments with diamines did not achieve the same effect. The idea of using Pd removal reagents in combination has been suggested previously (polymer-bound

(5) (a) Manley, P. W.; Acemoglu, M.; Marterer, W.; Pachinger, W. *Org. Process Res. Dev.* **2003**, *7*, 436. (b) Urawa, Y.; Miyazawa, M.; Ozeki, N.; Ogura, K. *Org. Process Res. Dev.* **2003**, *7*, 191. (c) Ragan, J. A.; Raggon, J. W.; Hill, P. D.; Jones, B. P.; McDermott, R. E.; Munchhof, M. J.; Marx, M. A.; Casavant, J. M.; Cooper, B. A.; Doty, J. L.; Lu, Y. *Org. Process Res. Dev.* **2003**, *7*, 676.

(6) Konisberger, K.; Chen, G.-P.; Wu, R. R.; Girgis, M. J.; Prasad, K.; Repic, O.; Blacklock, T. J. *Org. Process Res. Dev.* **2003**, *7*, 733.

(7) A number of alternatives were pursued for isolation of the crude Heck product. Precipitation directly from the reaction mixture gave product whose residual Pd content was either unusually high (>2500 ppm) or relatively low (~500 ppm), depending on the conditions used, compared to product isolated via the extractive workup. Elimination of residual Pd to below 20 ppm from **AG13736** isolated via any direct precipitation method tended to be more difficult.

(8) Purchased from Silicycle Inc., 1200 Ave St-Jean-Baptiste, Suite 114, Quebec City, Quebec, G2E 5E8, Canada (www.silicycle.com).

Table 3. **Optimization of basic parameters of the Pd removal process for AG13736**

entry[a]	NMP/MeOH ratio (v/v)	rxn. times (rxn./granulation)	1,2-Pn (wt equiv)	DPPE (wt equiv)	$Pd_{residual}$ [% removal]	recovery[b] (%)
1	10/20	18 h/18 h	1.0	0.2	26 ppm [98%]	N/A
2	10/20	5 h/18 h	1.0	−	43 ppm [98%]	65
3	10/20	6 h/18 h	1.0	−	83 ppm [93%]	49
4	10/20	5 h/18 h	1.0	−	84 ppm [93%]	49
5	10/20	18 h/18 h	1.0	−	26 ppm [98%]	N/A
6	10/20	12 h/12 h	1.0	−	30 ppm [99%]	79
7	10/20	12 h/12 h	−	1.0	124 ppm [96%]	78
8	10/20	12 h/12 h	0.5	0.5	31 ppm [99%]	78
9	10/20	19 h/70 h	0.5	0.5	8 ppm [99%]	83
10	10/20	2.5 h/15 h	0.5	0.2	16 ppm [85%]	75
11	10/20	18 h/36 h	0.5	0.2	17 ppm [99%]	61
12	10/20	2 h/18 h	0.5	0.2	26 ppm [98%]	64
13	10/20	2 h/2 h	0.5	0.2	19 ppm [99%]	52
14	10/20	18 h/2 h	0.5	0.2	21 ppm [98%]	56
15	5/10	2 h/18 h	0.5	0.2	39 ppm [97%]	76
16	5/20	2 h/18 h	0.5	0.2	28 ppm [98%]	76
17	5/40	2 h/18 h	0.5	0.2	17 ppm [99%]	83

[a] Entries 1, 3−5, and 9−17 used **AG13736** material containing 1189 ppm Pd. Entries 2, 6−8 used **AG13736** material containing 2807 ppm Pd. Reaction conditions: stirred at RT for time indicated, cooled, and granulated at RT for time indicated then filtered. [b] Nonpurity-corrected (some samples contaminated with DPPE).

ethylenediamine and trimercaptotriazine).[5b] A summary of results using the combination of 1,2-Pn and DPPE appears in Table 2.

Basic optimization was carried out in the weeks prior to scale-up to minimize the amounts of each reagent required to reduce the residual Pd content to below 20 ppm and to reduce the overall amount of reaction solvent and reaction time required. Since final yield was a critical parameter, we decided to pursue a process that used 5 vol of NMP for the Pd treatment step followed by addition of a relatively large quantity of MeOH to maximize recovery of crystallized product. As shown in Table 3, the addition of additional MeOH gave a modest bump in yield without suffering any significant increase in palladium content in the resulting filtered product. The "final" process was demonstrated once only at multigram scale (35 g) prior to transferring directly to the process plant.

The following operationally simplified procedure was carried out on 14.1 kg scale: a comixture of **AG13736** (87% HPLC purity, 326 ppm Pd), DPPE (0.2 wt equiv) and 1,2-Pn (0.5 wt equiv) in NMP (5 vol) was stirred at room temperature. After 2.5 h, methanol (40 vol) was charged, and the resulting slurry was allowed to continue stirring at room temperature for 18 h. Filtration of the resulting granular solids was very fast (<10 min) on a Comber filter dryer. The product thus obtained (10.0 kg, 96.5% HPLC purity, ~82% yield corrected for potency of the crude material) contained only 5 ppm residual palladium metal by inductively coupled plasma mass spectrometry (ICP-MS). The product was completely free of 1,2-Pn and DPPE by ^{1}H NMR. Two heated reslurries of the product in THF and in MeOH (10 vol in each case) were then conducted to remove chemical impurities remaining in the drug substance. Our intention in using a Comber filter dryer was to demonstrate a process-streamlining technique that could potentially be exploited with the new endgame process; since both purification and Pd removal could now be achieved while keeping the product out of solution (e.g., residual heavy metals and impurities are carried away in the mother liquor), this approach would be well-suited to the use of a Rosenmund-type (filtering) reactor on manufacturing scale. This strategy would allow all of the final operations to be carried out in a single reactor, minimizing time and resources. Unfortunately, an issue with the vapor condenser available to the Comber filter dryer at the time did not allow us to carry out heated reslurries in this vessel; however, it is ultimately the goal that Pd removal and final purification could be carried out over a maximum of two steps in a filtering reactor (see Figure 1 for a schematic representation). Final reslurrying steps led to final API material (99.73% HPLC purity) that met all purity specifications. This manufacturing campaign was the first time that both the second-generation synthesis and the new endgame purification process were conducted at large-scale for production of a GMP batch.

3.3. Results with Reslurry Techniques. After the successful scale-up run, it was decided to revisit the reslurry technique for palladium removal that had been initially tried and abandoned, using the new DPPE−1,2-Pn treatment. Using two different lots of **AG13736** (containing 326 and 1189 ppm Pd), each lot was suspended in 10 vol of 4:1 THF−MeOH and treated with 0.5 wt equiv of 1,2-Pn and 0.2 wt equiv of DPPE (19 h at 60 °C, 1 h at RT). Both filtered solids (>85% yield) were found to contain only 6 ppm residual Pd each. This result serves to further validate that both the recrystallization and reslurry can, in principle, be effective in this context. Another possible means for removal would be a liquid−liquid extraction where the soluble scavenging reagent would occupy the organic phase and **AG13736** would remain dissolved in an aqueous acidic phase. This approach would likely preclude the use of metal-coordinating substances that are largely protonated at pH 1−2 (e.g., diamines) but perhaps not others such as thiols or phosphines. This approach was not ultimately pursued, but the idea bears mentioning since this strategy could conceivably be incorporated as part of a workup for the Heck reaction, which may be pursued at a later date. The addition of DPPE into the process does add considerable cost, and future work on this process would likely be committed to greatly reducing or eliminating its role in the removal process. The exact reasons for the success of the ligand mixture over a single-reagent treatment are still not fully understood but a detailed study on

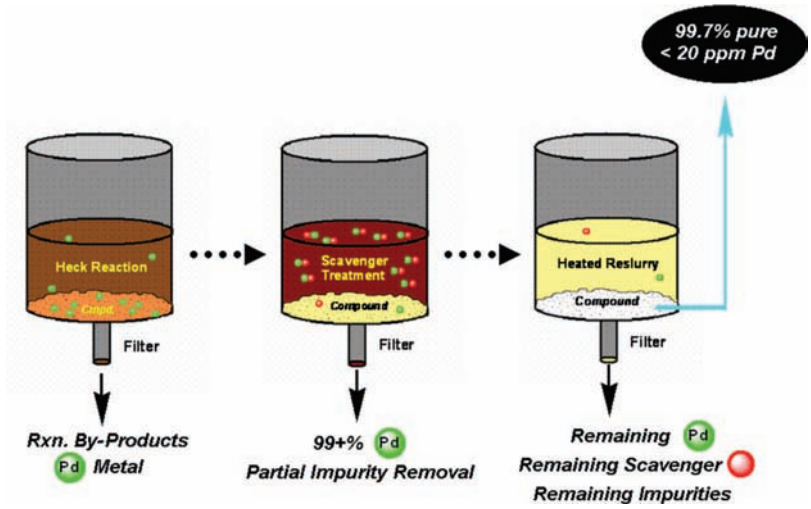


Figure 1. **Illustration of potential single-vessel approach to removal of residual Pd via ligand-assisted liquid−solid extraction.**

this effect is planned and will be reported in a separate communication.

3.4. Ligand-Screening Work and Results. During the course of our initial development work, the promising results obtained with diamine chelating agents led us to consider screening a broader range of potential metal scavengers with our in-house technology group. A novel approach for screening a range of adsorbents for removal of residual metals was recently described by Merck scientists in a past issue of *Org. Process Res. Dev.*[9] A similar strategy appeared well-suited for the desired ligand study, and our technology group began work to formulate high-throughput methodology to meet our needs. Additional consideration was given to the Pd analysis, since although screening hundreds of ligands may be plausible from the process technology perspective, the downstream Pd analysis may represent a potential bottleneck. The analytical team considered two options for analysis of residual Pd, ICP-MS and X-ray fluorescence (XRF), since both are potentially suited for high-throughput applications. Although XRF is less commonly used for analysis of liquid samples, the inadequate sensitivity of this method relative to that of ICP-MS proved the main factor in pursuing the latter technology. In addition, the ICP-MS method was identified as the better option since acid digestion utilized by this technique prior to analysis was considered necessary to ensure any ligated Pd was effectively analyzed. Using an in-house Perkin-Elmer SCIEX Elan DRC-II instrument with autosampling capabilities, a number of samples could be easily analyzed in automated sequence after acid digestion of a standard volume (50 μL) of each reaction supernatant. A screening design was built around the new conditions for Pd removal (ligand (0.5 wt equiv) in NMP, see Experimental Section), and the technology team applied a high-throughput workstream using both weighing and liquid-dispensing technologies. After treatment and addition of antisolvent, the reaction vials were centrifuged, and samples of the mother liquor were tested for residual Pd by ICP-MS. This protocol was used to screen 108 potential Pd scavengers in a single run. An analogous screen was also conducted using the optimal reslurry conditions (4:1 THF−MeOH). The screen successfully identified 10−15 ligands as most efficient for retention of Pd in the mother liquor. A more detailed account of the range of ligands tested in this study, as well as further work toward a computational approach to identification of other potential ligands, has been recently published.[10]

With a handful a potential leads having been identified by the ligand screen, our next step was to validate these results with a set of bench-scale experiments. For these tests, we chose to employ equimolar amounts of each ligand as opposed to weight equivalents in order to get a clearer picture of the comparative ability of each ligand to retain palladium in the mother liquor under the experimental conditions. The results are summarized in Table 4. The results confirmed that the large majority of hits from the screen were quite effective for Pd removal with most chelating agents removing greater than 85% of Pd metal in a single pass. The control experiment (entry 18) shows that appreciable clearance of palladium is also effected in the crystallization process itself. Nearly identical results were obtained when the NMP solution of **AG13736** was passed through a 0.2 μm filter before crystallization, suggesting that colloidal Pd metal is not present to any significant degree. The most effective compounds were diphosphine chelating agents (entries 12−14) which removed Pd to levels well below the desired specification of 20 ppm. DPPE and DPPP were more effective than DPPB for this purpose. Excellent results, as expected, were also obtained with the diamine reagents (entries 1−4); interestingly, 1,3-Pn appeared to be slightly better than diamine chelating agents containing an ethyl linker between heteroatoms. Also noteworthy was ethylene glycol which showed moderately efficient removal of palladium and would represent one of the more cost-effective options of the compounds tested (exposure issues notwithstanding). Overall the diamines remain the most practical solution in the context of a

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Table 4. **Bench-scale experiments[a] based on hits from ligand-screening work**

entry	scavenger[b] (3.2 mol equiv)	cost[c]	$Pd_{residual}$ [% removal][d]
1	1,2-En	$9/kg	48 ppm [87%]
2	1,2-Pn	$41/kg	42 ppm [88%]
3	1,3-Pn	$38/kg	33 ppm [91%]
4	TMEDA	$71/kg	33 ppm [91%]
5	ethylene glycol	$7/kg	61 ppm [83%]
6	2-aminophenol	$98/kg	54 ppm [85%]
7	catechol	$31/kg	58 ppm [84%]
8	1,2-benzenedithiol	>$5000/kg	26 ppm [93%]
9	thiourea	$28/kg	170 ppm [53%]
10	NAC	$1400/kg	N/A[e]
11	proton sponge	$2500/kg	41 ppm [89%]
12	DPPE	$1500/kg	5 ppm [99%]
13	DPPP	$5000/kg	5 ppm [99%]
14	DPPB	$1000/kg	17 ppm [95%]
15	bis[(*t*-Bu)$_2$P]-Cp$_2$Fe	>$5000/kg	N/A[f]
16	3,3-Me$_2$-glutaric acid	$1000/kg	75 ppm [79%][g]
17	4-ketopimelic acid	$5000/kg	155 ppm [48%][g]
18	(none)	−	92 ppm [75%]

[a] All experiments were run as follows: scavenger (4.2 mmol, 3.2 mol equiv) was added to a solution of 0.5 g of **AG13736** in 2.5 mL of NMP (5 vol), and the resulting mixture was stirred at room temperature overnight (18 h). A mixture of MeOH (20 vol) and water (3 vol) was then added, and stirring was continued for 72 h. Solids were collected by filtration, washed with 2 vol of MeOH, dried for 18 h at 60 °C, and then tested for residual Pd by ICP-MS. **AG13736** used contained 362 ppm Pd. [b] Scavenger abbreviations: *TMEDA* = *N*,*N*,*N*′,*N*′-ethylenediamine; *DPPE* = 1,2-bis(diphenylphoshino)ethane; *DPPP* = 1,3-bis(diphenylphoshino)propane; *DPPB* = 1,4-bis(diphenylphoshino)butane. [c] Approximate prices from Sigma-Aldrich. [d] ICP-MS, average of duplicate test results. [e] This experiment did not produce a solid. Attempts to induce precipitation via neutralization with Et_3N were unsuccessful. [f] Isolated solids were contaminated with bis[(*t*-Bu)$_2$P]-Cp$_2$Fe and decomposed on drying in vacuum oven at 60 °C. [g] Filtered product in each of these cases is a salt of **AG13736** with the acidic scavenger employed.

manufacturing process based on low cost, reduced toxicity concerns, and ease of removal from a final API, if present (based on the volatility of compounds such as 1,2-Pn versus nonvolatiles such as ethylene glycol). The availability of this data unfortunately did not precede the phase II manufacturing start date, but follow-up work is planned in due course. In a final series of bench-scale experiments, it was confirmed that excellent results (11 ppm residual Pd) could still be obtained after a single treatment with further reduced quantities of 1,2-Pn and DPPE (0.1 wt equiv each) used in combination. Further reduction of the reagent amounts to below 0.1 wt equiv was not attempted.

4. Conclusions

In summary, we have presented a process followed for rapid development of an efficient palladium removal method for the Axitinib API. Rapid, qualitative solubility screening can be effectively used to complement an approach where removal of residual palladium into a suitable solvent containing a metal-coordinating agent via either a recrystallization or reslurry strategy is planned. The ligand-screening method presented can further increase the efficiency of the development process. In our case, the new endgame process represents a >200-fold cost-savings improvement based on the cost of metal scavenger reagents alone (1,2-Pn/DPPE versus cysteine/silica) and further is a much greener process in terms of the overall solvents/raw materials used and the potential ease of recovery/recycling of palladium metal. We believe this is an approach that others could find useful for removal of heavy metals from pharmaceutical intermediates and APIs, particularly ones that are poorly soluble.

5. Experimental Section

5.1. Analytical Methods. HPLC purity was determined by a standard method using H_2O (0.1% trifluoroacetic acid)/ACN (0.1% trifluoroacetic acid) gradient on a Phenomenex Prodigy 100 × 4.6 mm (3 μm) column. Quantitative analysis of residual Pd was carried out via inductively coupled plasma mass spectrometry (ICP-MS) using a Perkin-Elmer SCIEX Elan DRC-II instrument. All samples were acid-digested (4.5% H_2SO_4 + 8.5% 3:1 HCl-HNO_3) with warming on a hotplate, and diluted to a standard volume prior to analysis.

5.2. Solvent Screening Study. The following work was carried out to determine the effect of solvent type on the efficiency of the liquid−solid extraction technique (reslurry) for removal of residual palladium and chemical impurities from crude **AG13736**. Solubility in a total of 32 unique solvents or solvent combinations was tested. Crude **AG13736** (10 mg) was suspended in 0.1 mL (10 vol) of the test solvent in a 96-vial plate setup. After stirring for 18 h at 20 °C, solids were settled via centrifugation and the mother liquor was analyzed by HPLC (3 min method). Based on the relative peak intensities for the product and reaction impurities, an impurity-purging efficiency score was assigned to each test solvent. Qualitative assessment of the color of each mother liquor (dark color relatively indicative of efficient metal-purging) was also carried out. These experiments were then repeated at 50 °C to generate data for heated reslurries. A number of favorable solvent mixtures were identified by these experiments and were reassessed in bench-scale experiments.

5.3. Palladium Removal Screening Method. A Mettler-Toledo FlexiWeigh solids-weighing robot was utilized to dispense eighty 50 mg aliquots of crude **AG13736** of known Pd contamination to 2 mL HPLC vials in a single overnight run. Following this solids dispense, NMP (5 mL/g, 0.25 mL) was added followed by the manually weighed ligands (0.5 wt equiv). A total of 108 ligands (commercially available amines, diamines, triamines, thiols, glycols, phosphines, etc.) were screened in a single run. Two control experiments were also conducted: an experiment with no antisolvent added (100% of residual Pd in the liquid phase) and an experiment where no ligand was added (Pd purging due to crystallization alone). A

simple heat cycle to 80 °C was carried out before the antisolvent mixture [MeOH (1 mL, 20 mL/g) and water (0.15 mL, 3 mL/g) was added. The mixture was granulated overnight. All reactions were then centrifuged leaving a large liquor volume from which to sample for acid digestion prior to ICP-MS analysis. Mother liquors containing the highest level of Pd relative to control runs were identified as the best hits for follow-up experiments. A detailed table of all ligands tested appears in a separate publication.[10]

5.4. Heck Reaction of Intermediate 5 with 2-Vinylpyridine (6). Preparation of Crude AG13736 (1). The following materials were charged to a glass-lined reactor via charge chute: compound **5** (20.2 kg, 49.4 mol), $Pd(OAc)_2$ (0.55 kg, 2.47 mol, 5 mol %), tri-*o*-tolylphosphine (2.0 kg, 6.42 mol, 13 mol %) and proton sponge (*N*,*N*,*N*′,*N*′-tetramethyl-1,8-napthalenediamine, 11.6 kg, 54.3 mol, 1.1 equiv). *N*-Methylpyrrolidone (NMP, 29 gal, 5.3 L/kg **5**) was charged, and the reactor was purged with nitrogen under stirring. Lithium bromide (26.6 kg, 306 mol, 6.2 equiv) was added via charge chute from an isolation chamber. The reactor was connected to a snorkel scrubber containing aqueous citric acid, and then 2-vinylpyridine (7.0 kg, 66.6 mol, 1.35 equiv) was charged to the reactor. The mixture was heated at 110 °C for 28 h, then cooled to 20 °C. To the mixture was added 1 N HCl_{aq} (55 gal) and methyl isobutyl ketone (MIBK, 55 gal). After stirring for 2 h, the mixture was filtered through Celite (to remove proton sponge salt), and the layers were separated. The organic layer was extracted with additional 1 N HCl (13 gal and 6 gal), which was combined with the original phase cut. The combined aqueous phase was washed with MIBK (27 gal), separated, and then combined with toluene (27 gal). To the resulting biphasic mixture, under vigorous stirring, was added 28 wt/wt % aqueous ammonium hydroxide (8 gal). The solids were collected by filtration on a Comber filter dryer and washed with water (27 gal) and toluene (11 gal). After drying under vacuum at 60 °C, the crude API[11] was obtained as a light-yellow solid (14.1 kg, 69%). Residual Pd metal content was 326 ppm, determined by ICP-MS. ^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm) 13.35 (1H, s), 8.61 (1H, d, J = 3.8 Hz), 8.39 (1H, q, J = 4.4 Hz), 8.21 (1H, d, J = 8.8 Hz), 7.96 (1H, d, J = 16.4 Hz), 7.85−7.76 (1H, m), 7.66 (1H, d, J = 7.8 Hz), 7.61 (1H, s), 7.58 (1H, d, J = 16.5 Hz), 7.50 (1H, dd, J = 5.7 Hz), 7.36−7.23 (3H, m), 7.19 (1H, dd, J = 8.4 Hz, 1.2 Hz), 7.05 (1H, dd, J = 7.5, 1.5 Hz), 2.78 (3H, d, J = 4.5 Hz). ^{13}C NMR (DMSO-d_6, 75 MHz) δ (ppm) 168.23, 155.18, 149.81, 142.35, 142.22, 137.31, 136.00, 132.89, 130.64, 129.51, 128.14, 126.50, 125.93, 124.08, 123.01, 122.85, 122.12, 120.64, 115.08, 26.45.

5.5. Manufacturing Process for Palladium Removal from Crude AG13736 (1). The following procedure was carried out using crude API material. Compound **1** (14.1 kg, 37 mol)) was charged via an isolation chute to a glass-lined reactor, followed by 2.9 kg (7.2 mol, 0.2 wt equiv) of 1,2-bis(diphenylphosphino)ethane. The reactor was purged with nitrogen and then charged with 19 gal of *N*-methylpyrrolidone (5 mL/g). The mixture was stirred at medium/low agitation while 1,2-diaminopropane (7 kg, 96.5 mol, 0.5 wt equiv) was added. The deep-red mixture was stirred at 20−30 °C for 2.5 h. Methanol (191 gal, 40 g/mL) was charged to the solution under medium agitation, and the resulting slurry was stirred at 20−30 °C for an additional 18 h. The product was collected on a Comber filter dryer dressed with a 5−7 μm nylon cloth. Filtration was complete within 15 min, and the resulting solid was rinsed with tetrahydrofuran (4 gal). Nitrogen was blown over the filter cake for 1 h, and the solids were taken to the next purification step without further drying (3.5% MeOH and 7% THF by GC headspace). The wet-cake product was a white solid (10.0 kg, 71% yield) containing 5 ppm of residual Pd by ICP-MS (after thorough vacuum oven drying of analytical sample). 1,2-Diaminopropane and 1,2-bis(diphenylphosphino)ethane were undetectable by ^{1}H NMR. HPLC purity = 96.5% (TFASH.M method).

5.6. Final Purification of AG13736 (1). The following steps were carried out to obtain drug substance of the requisite final chemical purity. The product obtained from the Pd-removal step (10.0 kg) was charged to a glass-lined reactor followed by tetrahydrofuran (26 gal, 10 mL/g). The reactor was purged with nitrogen, and agitation was set to medium. The heterogeneous mixture was heated at 60−70 °C for 8 h and then cooled to 20−30 °C and stirred for an additional 5 h. The product was collected on a Nutsche filter and washed with methanol (4 gal); nitrogen was blown over the wetcake for 1 h. The material thus obtained was 98.4% pure by HPLC (TFASH.M) and contained undetectable amounts of *N*-methypyrrolidone, 1,2-diaminopropane, and 1,2-bis(diphenylphosphino)ethane by ^{1}H NMR analysis. The wetcake material (9.0 kg) was charged back to the reactor, and methanol (23 gal, 10 mL/g) was added. The reactor was purged with nitrogen, and agitation was set to medium. The heterogeneous mixture was heated at 60−70 °C for 4 h and then cooled to 20−30 °C and stirred for an additional 4 h. The product was collected on a Nutsche filter, and nitrogen was blown over the wetcake for 1 h. This provided 7.8 kg of product that was 99.1% pure by HPLC (TFASH.M) and contained 0.6 ppm residual Pd metal by ICP-MS.

It is here noted that some issues arose during the scale-up the of final two reslurry steps (reslurry in 10 vol each of THF and MeOH at reflux) that required additional reslurries to remove higher-than-expected residual quantities of a known chemical impurity. These additional rework steps were not required in previous bench-scale experiments leading up to the pilot-plant manufacturing run. Repeat reslurries in MeOH (10 vol) and in THF (10 vol) provided clinical-grade drug substance (7.0 kg, 99.73% HPLC purity) that was submitted to the final polymorph control step.

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