Organic Process Research & Development

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Process Development, Impurity Control, and Production of a Novel Tubulin Inhibitor

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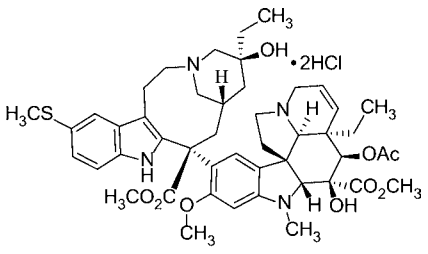
Supporting Information

ABSTRACT: Process development and production of a novel tubulin inhibitor are described. The desired API was obtained through selective iodination of the 12′ position of vinblastine and subsequent thiomethylation. Most of the impurities were identified, and process parameters were adjusted to control such impurities. The optimized process was scaled up under cGMP conditions to afford 230 g of the desired API.

1. INTRODUCTION

The vinca alkaloids have attracted significant attention in the field of cancer treatment since vinblastine and vincristine were first isolated from *Catharanthus roseus* in the late 1950s.[1−3] It is known that vinca alkaloids bind tubulin and inhibit microtubule formation, thus preventing cell mitosis and making them effective cancer chemotherapeutic agents especially for the treatment of leukemia and lymphoma patients.[4−6] Over the years a variety of vinca alkaloids have been obtained via total synthesis by several research groups.[7−16] However, structural modification of the natural products remains as an efficient and viable approach to obtain vinca derivatives. In the search for novel cancer chemotherapeutic agents, compound **1** (Figure 1)



1

Figure 1. Structure of **1**.

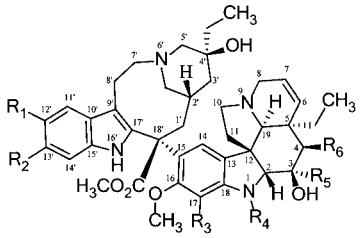
was identified as a potent tubulin inhibitor with potentially improved pharmacological and therapeutic properties.[17−19] During the discovery phase the active pharmaceutical ingredient (API) was obtained through iodination of vinblastine using *N*-iodosuccinimide (NIS) in 1:1 trifluoroacetic acid (TFA)/dichloromethane (DCM) and subsequent thiomethylation of the resulting 12′-iodovinblastine (**3**) in a sealed system. Many impurities were observed in each stage, and chromatographic purification was required for both intermediates. The desired salt form of the API was obtained by adding HCl to the freebase solution and by concentrating the mixture to dryness under reduced pressure. This salt formation method did not provide any purity upgrade, and purification by reverse phase chromatography was required to achieve the desired purity. It was clear that a viable process for large-scale manufacture of the bulk API would require careful control of impurity formation and removal. By means of identifying all the problematic impurities and determining the circumstances of their genesis, we ultimately derived a robust process that will reproducibly deliver multikilogram quantities of this drug. In this paper, we discuss our development of efficient impurity control for this challenging molecule, the process that resulted, and scale-up into the plant.

2. RESULTS AND DISCUSSION

2.1. Iodination. *2.1.1. Optimization of Iodination.* In the original discovery procedure, the conversion of vinblastine sulfate (**2**) to 12′-iodovinblastine (**3**) was performed in a 1:1 mixture of TFA/DCM at −20 to −15 °C.[20] This procedure suffered from low reproducibility and often low-quality material that was contaminated with 12′,13′-diiodovinblastine (**5**) (Figure 2) as the major impurity. Although this impurity was converted back to the desired product **3** by treatment with zinc dust in acidic ethanol (EtOH), chromatographic purification was still required to achieve acceptable purity (>95%).[21] Attempts to minimize the formation of **5** by changing cosolvents, adjusting the amount of TFA, or reducing reaction temperature were fruitless. Using the free base of vinblastine rather than the sulfate salt provided no advantage. Iodination with iodine monochloride (ICl) in the presence of indium(III) trifluoromethanesulfonate [In(OTf)$_3$] as a promoter[22] afforded

Received: August 17, 2012
Published: September 28, 2012

   dx.doi.org/10.1021/op3002307 | *Org. Process Res. Dev.* 2012, 16, 1811−1817



Impurity ID#	R_1	R_2	R_3	R_4	R_5	R_6
5	I	I	H	CH_3	CO_2CH_3	OAc
6	SCH_3	H	H	CH_3	CO_2CH_3	OH
7	SCH_3	SCH_3	H	CH_3	CO_2CH_3	OAc
8	SCH_3	H	I	CH_3	CO_2CH_3	OAc
9	H	I	H	CH_3	CO_2CH_3	OAc
10	H	SCH_3	H	CH_3	CO_2CH_3	OAc
11	SCH_3	H	Br	CH_3	CO_2CH_3	OAc
12	I	H	Br	CH_3	CO_2CH_3	OAc
13	SCH_3	H	H	CHO	CO_2CH_3	OAc
14	$S(O)CH_3$	H	H	CH_3	CO_2CH_3	OAc
15	SCH_3	H	H	CH_3	CO_2H	OAc

Figure 2. Structures of impurities.[24]

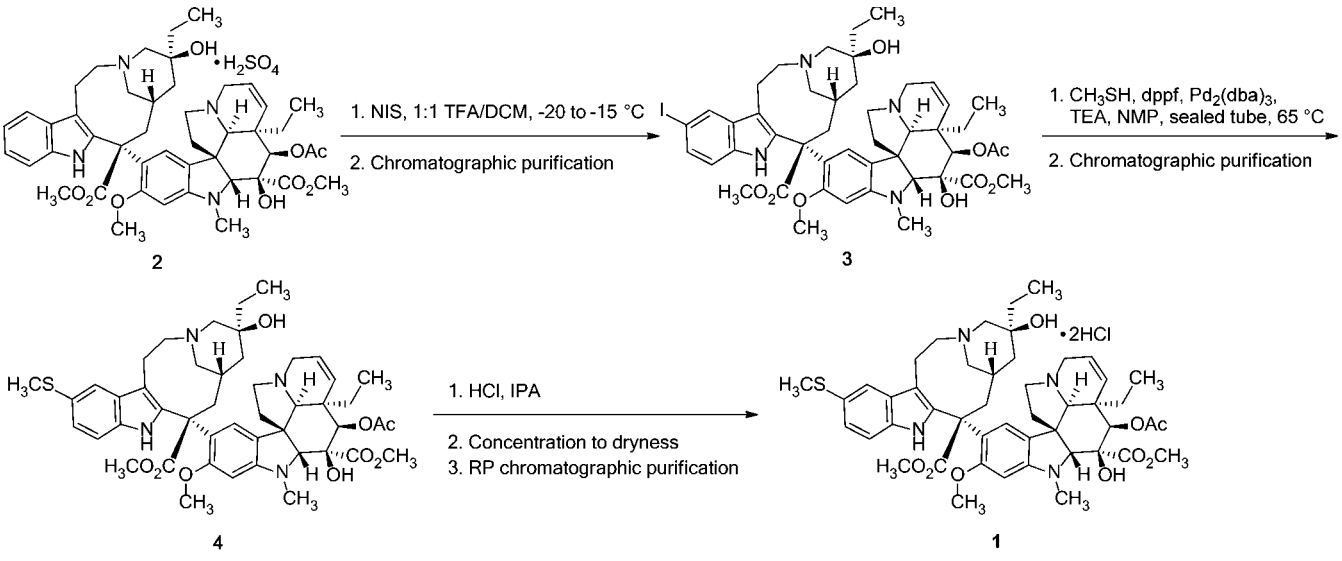
the desired product **3** in ~95% purity; however, significant levels of **5** (>3%) were still observed in this reaction. A much cleaner reaction profile was obtained when the iodination was performed with NIS in the presence of H_2SO_4 and acetic acid (AcOH) in acetonitrile (Scheme 1).[23]

Reduction in the amount of H_2SO_4 from 17 equiv to 2.1 equiv reduced the overall amount of unidentified impurities. This reduction in the amount of H_2SO_4 also resulted in less Na_2SO_4 being formed in the workup, which tended to precipitate and required a copious amount of water to dissolve. Reducing the amount of H_2SO_4 below 2.1 equiv resulted in a considerably slower reaction. Better selectivity was observed when the reaction temperature was maintained between −20 and −15 °C vs between −15 and −5 °C. Maintaining a lower concentration of NIS in the reaction mixture by slower NIS addition rates (over ~3 h) resulted in increased selectivity, thus, higher purities. When the temperature and/or the amount of acid were too low (<2 equiv H_2SO_4 or at <−20 °C), the reaction rate slowed to a point at which **2** was consumed more slowly than the NIS addition rate, which caused a relative increase in NIS concentration and ultimately lower selectivity and higher amounts of impurities. Further investigations revealed that using substoichiometric amounts of NIS (~0.95 equiv) at between −20 and −15 °C lowered the amounts of **5** and **2** (both <2.0%) that were present at the end of the reaction as a result of increased selectivity. Introduction of AcOH into the reaction system increased the overall purity and prevented precipitation of the vinblastine salt at low temperature (below −20 °C) before NIS addition.

An unknown impurity[25] was observed during the basic quench and workup. Additional studies showed that the formation of this impurity was suppressed when the reaction mixture was quenched by addition of $Na_2S_2O_3$ solution before being neutralized and extracted with a 10% Na_2CO_3/isopropyl acetate (IPAc) mixture. Instead of isolating and purifying the product by flash chromatography as reported in the original discovery procedure, we found that the crude product worked similarly well in the subsequent thiomethylation. The solvent of the organic layer was swapped for *N*-methylpyrrolidone (NMP) for use in the next stage.

2.1.2. Control of Iodination-Derived Impurities. Stability study indicated that the formation of **5** was reversible and followed an exponential decay profile as shown in Figure 3. On the basis of the results of the stability studies the optimal temperature for the iodination was found to be 5−10 °C which allowed for conversion of **5** to the desired product **3** without significant degradation to des-acetyl 12′-thiomethylvinblastine (**6**), after thiomethylation as observed at ambient temperature. Des-acetyl 12′-thiomethylvinblastine (**6**) was not a concern as it was generally purged to undetectable levels after the first recrystallization of **1**. 12′,13-Diiodovinblastine (**5**) was responsible for the formation of 12′,13′-dithiomethylvinblastine (**7**) observed after the thiomethylation reaction when the

Scheme 1. Original conditions for the synthesis of 1



dx.doi.org/10.1021/op3002307 | Org. Process Res. Dev. 2012, 16, 1811−1817

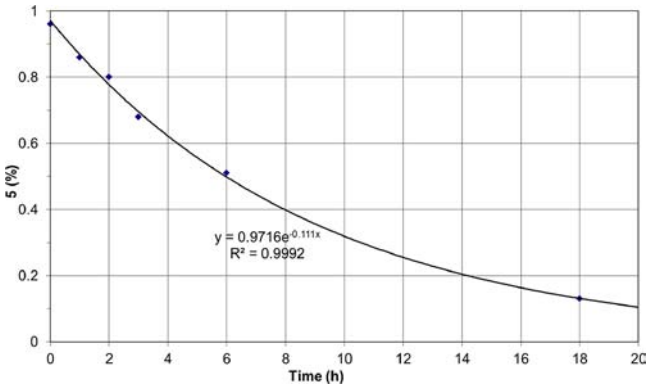


Figure 3. Reduction of **12′**,**13′**-diiodovinblastine, **5**, at 5−10 °C.

thiomethylation was performed at higher temperatures. 17-Iodo-12′-thiomethylvinblastine (**8**) instead of 12′,13′-dithiomethylvinblastine (**7**) was observed when using lower-temperature thiomethylation reaction conditions. During the conversion to the 12′-thiomethylvinblastine (**4**), 12′,13′-diiodovinblastine (**5**) was suspected to have behaved as an iodination reagent, resulting in several impurities, with **8** as the most predominant. Stirring the iodination reaction mixture at 5−10 °C after the reaction was complete significantly reduced the amount of **5** (to <0.5%) and thus provided a good control over **8** after thiomethylation.

Several other impurities observed in the final API were also tracked back to the iodination stage as there are multiple sites for iodination. Although the 12′ position is the most reactive site, iodination on other positions did occur. Iodination of the 13′ position of vinblastine [13′-iodovinblastine (**9**)] gave rise to 13′-thiomethylvinblastine (**10**) in the API after thiomethylation. This impurity was present in the crude samples of 12′-thiomethylvinblastine (**4**) at levels up to 3.5% during development studies; however, it was demonstrated that this impurity purged well during salt formation/recrystallization and was generally well below the specification limit (≤0.6%) after the second recrystallization.

17-Bromo-12′-thiomethylvinblastine (**11**) was traced to *N*-bromosuccinimide (NBS) contamination (∼0.4−1%) in NIS. This NBS contamination resulted in the formation of 17-bromo-12′-iodovinblastine (**12**) after the iodination, which was converted to similar amounts of **11** after thiomethylation and persisted at significant amounts (0.4%−0.8%) after subsequent recrystallizations. Using commercially available "NBS-free" NIS (contained <0.1% of NBS) resulted in almost undetectable amounts of this impurity in the IPC (<0.1%) that was not detected after further processing. The structure of **11** was later confirmed by comparison with an authentic sample obtained through chemical synthesis.[24] The charge of NIS must be adjusted to account for higher potency when using "NBS-free" NIS (higher wt% purity) in order to control the formation of 12′,13′-diiodovinblastine (**5**).

2.2. Thiomethylation. *2.2.1. Optimization of Thiomethylation.* Development work on the palladium catalyzed thiomethylation reaction began by reproducing the chemistry inherited from our Discovery colleagues. The reaction routely went to completion in a sealed system in NMP at 65 °C using 15 equiv of methanethiol (MeSH) in the presence of a combination of tris(dibenzylideneacetone)dipalladium(0) [$Pd_2(dba)_3$] and 1,1′-bis(diphenylphosphino)-ferrocene (dppf) with triethylamine (TEA) as the base;[26] however, incomplete reaction was observed when the reaction was run with a nitrogen sweep. Investigations revealed that the incomplete conversion was caused by loss of MeSH as a result of the nitrogen sweep. Further optimization revealed that the optimal amount of MeSH was 1.5 equiv as complete conversion was always achieved using this amount of MeSH, while incomplete conversion was observed when less than 1.2 equiv of MeSH was used.

Originally, 10 equiv of TEA was used in this reaction; this was successfully reduced to 2 equiv after optimization. Attempts to reduce the amount of catalyst from 10 mol % to 5 mol % still allowed the reaction to go to completion as long as the mixtures were sufficiently sparged of oxygen, but these resulted in higher levels of **2** (up to 2.2% after thiomethylation). Increasing the ratio of dppf/$Pd_2(dba)_3$ to 4:1 from 2.5:1 helped to reduce the amount of **2** that was formed (down to <0.6% after thiomethylation) without noticeable detriment to the overall purity of the crude product.

Reverse addition of the 12′-iodovinblastine (**3**) solution into the catalyst solution had the advantage of limiting the time that **3** was exposed to elevated temperatures, reducing its decomposition during the reaction. When a solution of **3** and TEA in NMP was added over 40 min to the dppf/$Pd_2(dba)_3$/MeSH mixture at 50−60 °C, complete conversion resulted, but with a higher level of **2** (2.38%) than a standard reaction using the same material (1.59%). Slow addition (40 min) of the solution of **3** and MeSH in NMP into the dppf/$Pd_2(dba)_3$/TEA mixture also resulted in elevated amounts of **2** (>3%). Much lower levels of **2** (1.71%) were observed when the solution of **3** and MeSH was added over only 10 min. To minimize the loss of MeSH and deactivation of catalysts, TEA, MeSH, and dppf/$Pd_2(dba)_3$ were added in sequence over 10 min after the solution of **3** in NMP was heated to the desired temperature (50−60 °C). Although vinblastine (**2**) was observed at up to 2.5% in crude **4**, it was demonstrated that it could be purged to below the specification after the second recrystallization of **1**.

The crude product was isolated by filtration after aqueous workup, extraction with IPAc, and precipitation with heptane. Attempts to further purify crude **4** by recrystallization or reslurry were fruitless; consequently, efforts were focused on the purification by salt formation and recrystallization as discussed in the following section. The optimized inodination/thiomethylation process was demonstrated on a 115-g scale of vinblastine sulfate and afforded the desired product **4** in the expected purity (>90%).

2.2.2. Pd Removal. Crude **4** typically contained 500−1500 ppm Pd, which was reduced to around 100−200 ppm after the salt formation and two recrystallizations. In order to reduce residual Pd to an acceptable level, several Pd scavengers[27] were evaluated (Table 1) to treat the crude IPAc solution before concentration and precipitation. Among these, DARCO KB-G afforded the most significant reduction in the palladium content to 20−40 ppm after filtration to remove the scavenger and isolation of the product by precipitation. It was found that stirring for a longer time (entries 12 and 13), using additional scavenger (entry 13), and performing a second slurry (entry 14) all improved the sequestering of the residual Pd, although extended stirring time (∼18 h) had a slightly detrimental effect on the purity of the product. Further stability studies revealed that the purity was not affected when the mixture was stirred for 4 h or less.

dx.doi.org/10.1021/op3002307 | *Org. Process Res. Dev.* 2012, 16, 1811−1817

Table 1. Screen of scavengers

entry	scavenger	loading (wt%)	time (h)	Pd cotent after treatment (ppm)
1[a]	None	---	18	549
2	Darco-G60 100 mesh	10	18	39
3	Darco-KB-G	10	18	22
4	Trithiocyanuric acid	10	18	269
5	Ethylenediamine, polymer-bound	10	18	347
6	Smopex-111pp	10	18	404
7	Smopex-112pp	10	18	343
8	Smopex-234pp	10	18	263
9	PL-TMT MP Resin	10	18	29
10	Darco-KB-G	10	1	53
11	Darco-KB-G	10	4	28
12	Darco-KB-G	10	24	20
13	Darco-KB-G	20	24	12
14	Darco-KB-G	2 × 10	2 × 1	17

[a]Blank experiment.

2.2.3. Control of Thiomethylation Derived Impurities. 12′-Iodovinblastine (**3**) was one of the impurities that had been observed in the crude **4** when the thiomethylation did not go to completion. It was difficult to purge during the normal processing and additional recrystallizations of the API were found to slightly increase the amount of this impurity (from 0.3% to 0.5% after the salt formation and two recrystallizations) due to its poor solubilty in the recrystallization solvents. It was ultimately determined that under the standard reaction conditions the reaction would go to completion if good quality nitrogen (or argon) is used; poor quality (contaminated with oxygen) could result in incomplete conversion. Implementation of a reaction completion specification (≤0.1%) for the thiomethylation reaction helped to ensure that acceptable levels of **3** were achieved in the final API.

12′-Thiomethylvincristine (**13**) was generated from residual vincristine in starting material vinblastine (**2**). This impurity was not purged during any of the operations or purifications; however, it did not increase significantly through the process and was only found at low levels in the API (≤0.2%). 12′-Thiomethylvinblastine-S-oxide (**14**) and des-methyl 12′-thiomethylvinblastine (**15**) were observed respectively in up to 0.7% and 1.2% in **4**; nonetheless, they were purged down to 0.15% (specification ≤0.5%) and nondetectable (specification ≤0.5%), respectively, after the second recrystallization of the final API **1**.

2.3. Salt Formation and Recrystallization. Preliminary salt screening investigations revealed that the dihydrochloride salt of **4** was the most crystalline of the salts evaluated. However, the original procedure involved the addition of aq HCl and concentration to dryness to obtain the desired product **1**. This practice did not provide any upgrade in purity and was clearly not a scalable process. It was determined that preparing the salt in IPA by addition of 2.1 equiv of 2 M HCl in IPA resulted in a significant purity boost (89.0% to 92−94% purity) in >90% recovery. This condition was further optimized to improve volume efficiency and successfully applied to the production.

Preliminary results indicated that IPA/water was a good solvent system for recrystallization, increasing the API purity from 91% to 95% purity after the first recrystallization and to >95% purity after the second recrystallization (entry 1, Table 2). However, decomposition was observed during stability study, and attempts to modify this process to avoid degradation were unsuccessful. Several additional solvent systems were investigated, and similar decomposition was observed when water was present in the recrystallization matrix. Among all the screened solvents, MTBE/MeOH afforded the best purity with no decomposition during stability studies in albeit relatively poor recovery (48%) (entry 2). Subsequent studies showed that the recovery can be improved to ∼70% when IPA was introduced as an antisolvent without erosion of API purity (entries 4−6). This process was successfully demonstrated to afford the desired API in expected purity (>97%) with no noticeable degradation observed (see Scheme 2).

2.4. cGMP Production. Upon the completion of a successful demonstration batch, the first iodination of vinblastine for cGMP production went as expected to afford the crude 12′-iodovinblastine (**3**) as a NMP solution in expected purity and yield; however, a major impurity (>30% by HPLC, **16** in Figure 4) was observed in the thiomethylation stage that caused significant yield loss. The structure of **16** was assigned on the basis of LC/MS and LC/NMR data. Small-scale experiments revealed that using a deficit amount of MeSH (∼0.7 equiv) in the reaction resulted in the formation of this impurity, indicating that **16** was a direct result of MeSH loss from the reaction matrix caused by a slight vacuum applied to the laboratory's scrubber system. Insufficient amounts of MeSH promoted the reaction of the aryl palladium intermediate with the dibenzylidene acetone (dba) in a potentially combined Heck/Wacker-type reaction to give this major impurity along with many other unknown impurities. In addition, some sticky, dark, clumpy material precipitated during the brine washes. Although significant amounts of impurities were removed after isolation of **4**, several late-eluting impurities were still present, and the isolated material contained more than 5000 ppm of palladium compared to the typical ∼200 ppm level.

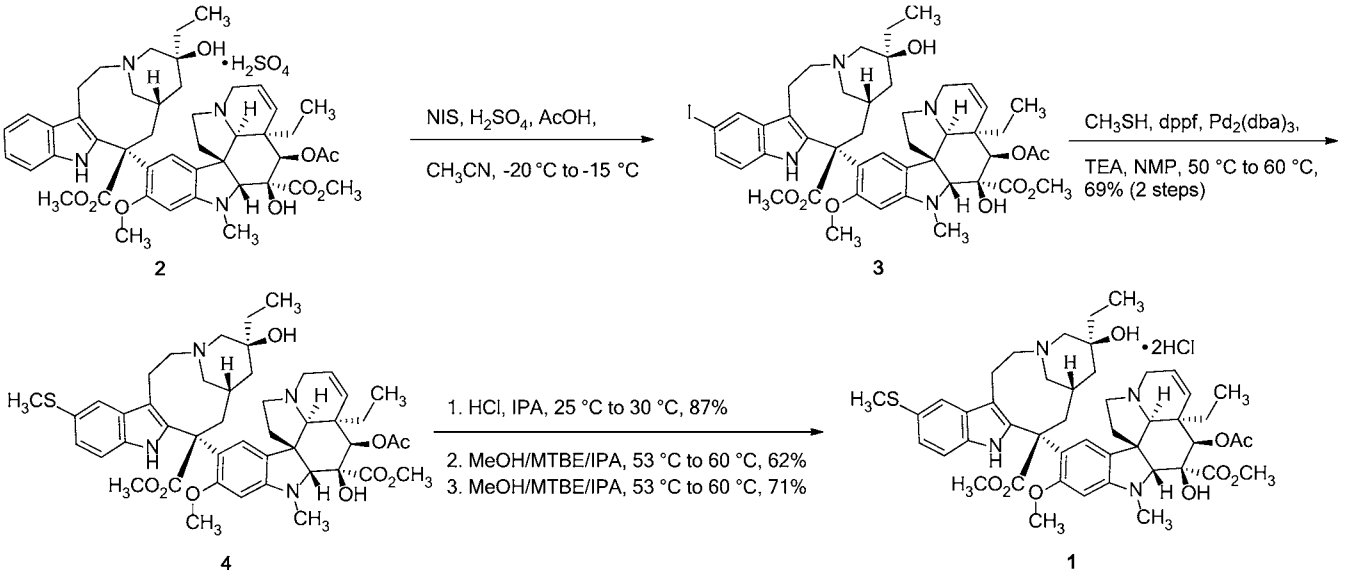
A portion of this material was carried forward to salt formation and two subsequent recrystallizations as a use-test. During the salt-formation stage a significant amount of material did not dissolve in IPA before addition of HCl. Polish filtration was necessary to remove the solid prior to the addition of HCl

Table 2. Recrystallization studies for 1

			1st recrystallization			2nd recrystallization		
entry	solvent system	starting purity	Scale	recovery	purity	scale	recovery	purity
1	IPA/H_2O	94.6%	218 g	83%	96.1%	167 g	69%	96.4%
2	MTBE/MeOH	92.0%	2.60 g	48%	96.5%	497 mg	53%	97.6%
3	IPA/MeOH	92.0%	2.44 g	71%	96.0%	520 mg	68%	97.7%
4	IPA/MTBE/MeOH	92.0%	953 mg	68%	95.9%	488 mg	68%	97.2%
5	IPA/MTBE/MeOH	92.0%	10.0 g	69%	96.0%	6.62 g	69%	97.6%
6	IPA/MTBE/MeOH	94.4%	526 g	62%	97.3%	324 g	72%	98.5%

dx.doi.org/10.1021/op3002307 | Org. Process Res. Dev. 2012, 16, 1811−1817

Scheme 2. Optimized conditions for the synthesis of 1



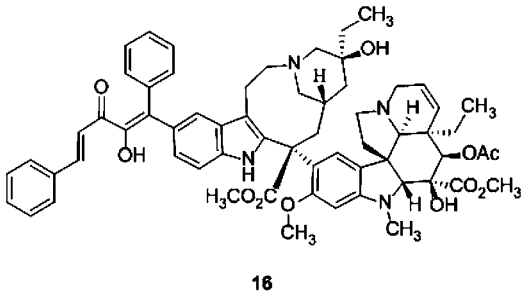


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Figure 4. Structure of the major impurity **16** observed in cGMP production.

which caused a very low recovery (61% vs 87%) after salt formation. The recrystallizations also had low recoveries (29% and 40%, respectively) and were unsuccessful in removing the late-eluting impurities and residual palladium. This batch of material was discarded.

On the basis of the observation of the first cGMP campaign, it was decided to use an ambient pressure scrubbing system under static conditions (no nitrogen flow) instead of an active scrubbing system to avoid removing MeSH before the catalyst was added. A test (^{1}H NMR in pyridine) was also put in place to verify that the concentration of MeSH in NMP was appropriate before use. Instead of using nitrogen pressure for the addition of the MeSH solution, the solution was directly added into the batch to speed the addition and prevent loss of MeSH from the reaction mixture.

Following this new procedure, two batches of crude **4** were obtained in consistent yield (69% and 65%) and purity (91.2% and 91.4%). The two batches of crude **4** were combined and submitted to the salt formation conditions to afford the desired crude product **1** in 87% yield and 94.4% purity. This material was recrystallized twice from MeOH/MTBE/IPA to afford 230 g of the desired API in 98.5% purity with all impurities controlled at below specifications (Table 3).

3. CONCLUSIONS

A robust process for the synthesis of a novel tubulin inhibitor **1** was developed. The target API was realized via selective iodination of vinblastine **2**, followed by a palladium catalyzed thiomethylation of 12′-iodovinblastine (**3**), salt formation, and recrystallization. Most impurities were identified and controlled by adjusting process parameters. An unexpected impurity was observed during the first production batch as a result of the reaction of 12′-iodovinblastine (**3**) with the catalyst due to loss of MeSH. Modifications to the process were incorporated in the second campaign, which successfully afforded the final API meeting all specifications.

Table 3. Impurity control during production

		crude free base				
impurity	specification	batch 1	batch 2	crude salt	1st recryst.	2nd recryst.
2	NMT 0.75%	1.5%	1.5%	1.3%	0.62%	0.35%
3	NMT 0.5%	0.07%	0.07%	0.06%	0.07%	0.07%
6	NMT 0.2%	0.26%	0.35%	0.17%	ND	0.04%
7	NMT 0.2%	ND	ND	ND	ND	ND
8	NMT 0.3%	0.18%	0.20%	0.19%	0.13%	0.07%
10	NMT 0.6%	ND	ND	ND	ND	ND
11	NMT 0.6%	ND	ND	ND	ND	ND
13	NMT 0.5%	0.18%	0.21%	0.20%	0.21%	0.23%
14	NMT 0.5%	ND	ND	ND	ND	ND
15	NMT 0.5%	ND	ND	ND	ND	ND

4. EXPERIMENTAL SECTION

Reaction progress and chemical purity were evaluated by HPLC analysis using a Cadenza CD-C18 column (150 × 4.6 mm, 3 μm) using the methods listed below. Method A: mobile phase A: water +0.1% TFA; mobile phase B: acetonitrile +0.1% TFA; detection: 215 nm; flow: 1.0 mL/min; column temp.: 40 °C; gradient: 0 min: A = 70%, B = 30%; 19 min: A = 55%, B = 45%; 24 min: A = 10%, B = 90%; 25 min: A = 70%, B = 30%; and 34 min: A = 70%, B = 30%. Method B: mobile phase A: water +0.1% TFA; mobile phase B: acetonitrile +0.1% TFA; detection: 234 nm; flow: 1.0 mL/min; column temp.: 40 °C; gradient: 0 min: A = 70%, B = 30%; 19 min: A = 55%, B = 45%; 24 min: A = 10%, B = 90%; 25 min: A = 70%, B = 30%; and 34 min: A = 70%, B = 30%. Method C: mobile phase A: water +0.1% TFA; mobile phase B: acetonitrile +0.05% TFA; detection: 215 nm; flow: 1.0 mL/min; column temp.: 40 °C;

dx.doi.org/10.1021/op3002307 | Org. Process Res. Dev. 2012, 16, 1811−1817

gradient: 0 min: A = 70%, B = 30%; 19 min: A = 55%, B = 45%; 24 min: A = 10%, B = 90%; 30 min: A = 10%, B = 90%; 31 min: A = 70%, B = 30%; 40 min: A = 70%, B = 30%.

4.1. 12′-Iodovinblastine (3). A solution of vinblastine sulfate (**2**) (0.47 kg, 0.52 mol) in acetonitrile (2.2 kg) was cooled to ≤−15 °C. H_2SO_4 (0.1 kg) was added over 40 min while maintaining the batch temperature between −20 and −15 °C. AcOH (0.12 kg) was added over 6 min while maintaining the batch temperature between −20 and −15 °C. A solution of NIS (111 g, 0.49 mol) in acetonitrile (1.33 kg) was added over 3 h while maintaining the batch temperature between −20 and −15 °C (0.3 kg of acetonitrile was used as a rinse). The reaction was stirred for 4 h, at which time HPLC analysis indicated that the reaction was incomplete with 1.3% of **2** remaining (Method A, specification: ≤1.0% of vinblastine). Additional NIS (1 g, 0.004 mol) was added while maintaining the batch temperature between −20 and −15 °C and the reaction was stirred for 70 min, at which point HPLC analysis indicated that the reaction was complete with 0.86% of **2** remaining (Method A). The reaction was adjusted to a temperature of 5−10 °C over 6 h and stirred for 32 h, at which point HPLC analysis indicated that only 0.53% of **5** remained (Method B, specification: ≤0.5% of **5**). A 3 wt % aqueous $Na_2S_2O_3$ solution (2.4 kg) was added and the reaction mixture was stirred for 2 h at 15−25 °C. The mixture was transferred through a polish filter into a mixture of 10 wt % Na_2CO_3 solution (7.1 kg) and IPAc (4.1 kg). The layers were separated and the organic layer was washed with water (2.4 kg). NMP (2.44 kg) was added and the batch was distilled at <40 °C under reduced pressure to ∼2.7 L. This solution was used directly in the thiomethylation stage without isolation. An analytical sample was obtained via purification by column chromatography: ^{1}H NMR (500 MHz, CD_3OD) 7.77 (d, 1 H, J = 1.5 Hz), 7.33 (dd, 1 H, J_1 = 2.0 Hz, J_2 = 8.5 Hz), 7.03 (d, 1 H, J = 8.5 Hz), 6.60 (s, 1 H), 6.33 (s, 1 H), 5.85 (ddd, 1 H, J_1 = 1.0 Hz, J_2 = 5.0 Hz, J_3 = 10.0 Hz), 5.50 (s, 1 H), 5.38 (s, 1 H), 5.31 (d, 1 H, J = 10.0 Hz), 4.06 (dd, 1 H, J_1 = 13.5 Hz, J_2 = 15.0 Hz), 3.95 (dd, 1 H, J_1 = 10.0 Hz, J_2 = 15.0 Hz), 3.83 (s, 3 H), 3.78 (s, 3 H), 3.65 (s, 3 H), 3.60 (s, 1 H), 3.39 (d, 1 H, J = 14.5 Hz), 3.32−3.19 (m, 4 H), 2.96 (dd, 1 H, J_1 = 5.5 Hz, J_2 = 15.0 Hz), 2.84 (d, 1 H, J = 14.5 Hz), 2.80−2.73 (m, 6 H), 2.69 (s, 2 H), 2.48 (dd, 1 H, J_1 = 4.0 Hz, J_2 = 14.5 Hz), 2.44−2.40 (m, 1 H), 2.30 (dd, 1 H, J_1 = 4.0 Hz, J_2 = 14.5 Hz), 2.13−2.08 (m, 1 H), 2.04 (s, 3 H), 1.92−1.85 (m, 1 H), 1.72−1.65 (m, 1 H), 1.53 (d, 1 H, J = 14 Hz), 1.48−1.37 (m, 2 H), 1.33 (q, 2 H, J = 7.5 Hz), 0.91 (t, 3 H, J = 7.5 Hz), 0.86−0.78 (m, 1 H), 0.78 (t, 3 H, J = 7.5 Hz); ^{13}C NMR (125 MHz, CD_3OD) 181.5, 177.0, 173.2, 172.5, 159.7, 154.3, 135.8, 133.5, 133.1, 131.5, 131.3, 128.1, 125.8, 125.4, 124.1, 122.5, 117.4, 114.1, 95.3, 84.3, 82.7, 81.4, 77.7, 69.6, 66.4, 64.2, 57.5, 57.1, 56.6, 54.7, 53.0, 52.9, 51.4, 50.9, 48.0, 45.9, 44.1, 41.4, 38.9, 35.9, 32.0, 30.6, 28.2, 21.0, 9.0, 7.3; HRMS calculated for $C_{46}H_{58}N_4O_9I$ (M + H): 937.3249, found: 937.3280; HPLC (Method A, t_R = 15.8 min): 95.8%;

4.2. 12′-Thiomethylvinblastine (4). The above obtained crude 12′-iodovinblastine (**3**) solution (∼2.7 L) was heated to 50−60 °C while sparging with N_2 for 30 min. TEA (160 g, 1.58 mol), a MeSH (42 g, 0.88 mol) solution in NMP (0.24 kg), and a $Pd_2(dba)_3$ (0.036 kg, 39 mmol)/dppf (0.086 g, 155 mmol) solution in NMP (1.45 kg) were added sequentially over 10 min. The reaction was stirred at 50−60 °C for 2 h, at which point HPLC analysis indicated that the reaction was complete (Method C, specification: ≤0.1% of **3**). The mixture was cooled to 20−30 °C, diluted with IPAc (4.3 kg), and washed with 14 wt % NaCl solution (2 × 7.2 kg). The organic layer was extracted with 0.5 M H_2SO_4 (5.69 kg) and water (2.4 kg). The combined extracts were diluted with IPAc (4.1 kg) and basified with 20% Na_2CO_3 solution (2.9 kg). After mixing, the layers were separated and the organic layer was washed with a 14 wt % NaCl solution (2.4 kg). Na_2SO_4 (0.5 kg) and DARCO KB-G (50 g) were added to the organic layer and the resulting suspension was stirred at 20−25 °C for 3.5 h. The mixture was filtered through a diatomaceous pad (0.5 kg) and the filtrate was concentrated to ∼4 L/kg relative to the input of **2**. The resulting solution was added into heptane (8.2 kg) over 35 min at 20−25 °C. The resulting suspension was stirred at 20−25 °C for 2 h and filtered. The filter cake was rinsed with heptane (0.97 kg), conditioned for 3 h, and dried under vacuum at 20−25 °C to afford the desired product **4** as an off-white solid [0.31 kg, 69% yield, HPLC (Method A, t_R = 13.5 min): 91.2%]. An analytical sample was obtained via purification by column chromatography: ^{1}H NMR (500 MHz, CD_3OD) 7.47 (s, 1 H), 7.14 (d, 2 H, J = 1.0 Hz), 6.62 (s, 1 H), 6.34 (s, 1 H), 5.85 (ddd, 1 H, J_1 = 1.0 Hz, J_2 = 5.0 Hz, J_3 = 10.5 Hz), 5.39 (s, 1 H), 5.31 (d, 1 H, J = 10.5 Hz), 4.07 (dt, 1 H, J_1 = 1.5 Hz, J_2 = 14.0 Hz), 3.96 (dd, 1 H, J_1 = 10.0 Hz, J_2 = 15.0 Hz), 3.83 (s, 3 H), 3.78 (s, 3 H), 3.65 (s, 3 H), 3.61 (s, 1 H), 3.42 (d, 1 H, J = 14.0 Hz), 3.30−3.19 (m, 4 H), 3.02 (dd, 1 H, J_1 = 6.0 Hz, J_2 = 15.0 Hz), 2.85 (d, 1 H, J = 13.5 Hz), 2.80−2.74 (m, 3 H), 2.73 (s, 3 H), 2.50−2.40 (m, 2 H), 2.45 (s, 3 H), 2.31 (dd, 1 H, J_1 = 4.0 Hz, J_2 = 16.0 Hz), 2.14−2.08 (m, 1 H), 2.04 (s, 3 H), 1.93−1.86 (m, 1 H), 1.74−1.68 (m, 1 H), 1.53 (d, 1 H, J = 14.0 Hz), 1.46−1.36 (m, 2 H), 1.32 (q, 2 H, J = 7.5 Hz), 0.92 (t, 3 H, J = 7.5 Hz), 0.87−0.82 (m, 1 H), 0.80 (t, 3 H, J = 7.5 Hz); ^{13}C NMR (125 MHz, CD_3OD) 177.1, 173.3, 172.5, 159.8, 154.3, 135.6, 133.1, 131.3, 131.1, 128.3, 125.8, 125.7, 125.4, 124.1, 122.7, 120.7, 117.7, 112.4, 95.4, 84.4, 81.3, 77.7, 69.6, 66.6, 64.2, 57.5, 57.1, 56.6, 54.7, 52.9, 52.8, 51.4, 51.0, 47.9, 45.8, 44.1, 41.4, 39.0, 35.9, 32.0, 30.9, 28.3, 21.0, 19.3, 9.0, 7.3; HRMS calculated for $C_{47}H_{61}N_4O_9S$ (M + H): 857.4159, found: 857.4161; HPLC (Method A, t_R = 13.5 min): 97.2%.

4.3. 12′-Thiomethylvinblastine·2HCl (1). A mixture of 12′-thiomethylvinblastine (**4**) (0.56 kg, 0.60 mmol) and IPA (4.4 kg) was heated to 20−30 °C to afford a clear solution. It was clarified using a polish filter and the filter was rinsed with IPA (0.44 kg). The combined filtrates were heated at 20−30 °C and 2 M HCl in IPA (0.59 kg, 2.1 equiv) was added over 1 h at 20−30 °C. The resulting suspension was stirred at 20−30 °C for 2.5 h and filtered. The filter cake was rinsed with IPA (2.11 kg), conditioned for 30 min, and dried under vacuum to constant weight to afford the crude product **1** as an off-white solid [0.53 kg, 87% yield, HPLC (Method A, t_R = 13.5 min): 94.4%]. The above obtained crude product was suspended in MTBE (1.18 kg)/MeOH (1.26 kg) and heated to 53−60 °C. MeOH (1.66 kg) was added over 100 min while maintaining the batch temperature at 53−60 °C. IPA (2.1 kg) was added over 35 min while maintaining the batch temperature at 53−60 °C and the resulting suspension was cooled to 0−5 °C over 4 h. The suspension was stirred at 0−5 °C for 70 min and filtered. The filter cake was rinsed with IPA (2 × 2.1 kg) and MTBE (1.9 kg), conditioned for 30 min, and dried under vacuum at 35−40 °C to constant weight to afford an off-white solid [0.32 kg, 62% yield, HPLC (Method A, t_R = 13.5 min): 97.3%]. The resulting solid was suspended in MTBE (0.72 kg)/MeOH (0.77 kg) and heated to 53−60 °C. MeOH (1.0 kg) was added over 30 min while maintaining the batch temperature at 53−60

dx.doi.org/10.1021/op3002307 | Org. Process Res. Dev. 2012, 16, 1811−1817

°C. IPA (1.3 kg) was added over 55 min while maintaining the batch temperature at 53−60 °C and the resulting suspension was cooled to 0−5 °C over 4 h. The suspension was stirred at 0−5 °C for 60 min and filtered. The filter cake was rinsed with IPA (2 × 1.3 kg) and MTBE (1.2 kg), conditioned for 30 min, and dried under vacuum at 35−40 °C to constant weight to afford the desired product **1** as an off-white solid (0.23 kg, 71% yield). Mp (DSC): 236 °C; ^{1}H NMR (500 MHz, CD_3OD) 9.69 (bs, 1 H), 7.43 (d, 1 H, J = 1.5 Hz), 7.13 (d, 1 H, J = 8.5 Hz), 7.05 (dd, 1 H, J_1 = 1.5 Hz, J_2 = 8.0 Hz), 6.58 (s, 1 H), 6.31 (s, 1 H), 5.84 (ddd, 1 H, J_1 = 1.0 Hz, J_2 = 5.0 Hz, J_3 = 10.0 Hz), 5.56 (dd, 1 H, J_1 = 1.5 Hz, J_2 = 10.5 Hz), 5.23 (s, 1 H), 4.52 (ddd, 1 H, J_1 = 2.0 Hz, J_2 = 10.5 Hz, J_3 = 15 Hz), 3.85−3.73 (m, 4 H), 3.75 (s, 3 H), 3.72 (s, 3 H), 3.72−3.70 (m, 1 H), 3.70−3.64 (m, 1 H), 3.63 (s, 1 H), 3.58 (s, 3 H), 3.58−3.50 (m, 2 H), 3.39 (d, 1 H, J = 16 Hz), 3.24−3.18 (m, 1 H), 3.05 (s, 2 H), 2.77 (dd, 1 H, J_1 = 6.5 Hz, J_2 = 14.5 Hz), 2.68 (s, 3 H), 2.34 (s, 3 H), 2.36−2.30 (m, 1 H), 2.28−2.22 (m, 1 H), 1.97 (s, 3 H), 1.98−1.90 (m, 1 H), 1.66−1.58 (m, 1 H), 1.54 (d, 2 H, J = 5.5 Hz), 1.49−1.43 (m, 1 H), 1.41 (q, 2 H, J = 7.5 Hz), 1.29−1.22 (m, 1 H), 1.04 (d, 2 H, J = 6.0 Hz), 0.86 (t, 3 H, J = 7.5 Hz), 0.68 (t, 3 H, J = 7.5 Hz); ^{13}C NMR (125 MHz, CD_3OD) 176.1, 172.1, 171.7, 160.5, 154.3, 136.3, 133.4, 132.8, 130.7, 129.2, 126.0, 125.0, 122.3, 121.0, 120.0, 114.4, 113.4, 95.5, 81.4, 81.2, 76.0, 68.4, 67.3, 64.8, 62.1, 57.9, 56.7, 53.9, 53.5, 53.1, 50.7, 50.1, 46.8, 44.7, 44.2, 38.4, 37.6, 36.5, 35.5, 32.1, 27.8, 25.3, 21.8, 20.8, 19.2, 8.4, 7.0; HRMS calculated for $C_{47}H_{61}N_4O_9S$ (free base + H): 857.4159, found: 857.4192; HPLC (Method A, t_R = 13.5 min): 98.5%; Karl Fisher: 1.2% water; Pd: 22 ppm.

■ ASSOCIATED CONTENT

Supporting Information

HRMS, MS/MS, ^{1}H NMR, COSY NMR, and HSQC NMR of impurity **16**. 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.

■ ACKNOWLEDGMENTS

We are grateful to our drug discovery colleagues for their support during this program. We thank our colleagues in the analytical group for their invaluable support in method development, the structure identification group for their assistance in identifying the impurities, and the AMRI RLS team members for the production of this project.

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