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Discovery and Development of an Efficient, Scalable, and Robust Route to the Novel CENP-E Inhibitor GSK923295A

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Abstract:

The discovery and development of an efficient manufacturing route to the CENP-E inhibitor 3-chloro-*N*-{(1*S*)-2-[(*N*,*N*-dimethylglycyl)amino]-1-[(4-{8-[(1*S*)-1-hydroxyethyl]imidazo[1,2-*a*]pyridin-2-yl}phenyl)methyl]ethyl}−4-[(1-methylethyl)oxy]benzamide (GSK923295A) is described. The existing route to GSK923295A was expensive, nonrobust, used nonideal reagents, and consistently struggled to deliver the API needed for clinical studies. The new synthesis commences from the readily available L-phenylalaninol, which is smoothly converted through to GSK923295A using key Friedel−Crafts acylation as well as selective acylation chemistries. Downstream chemistry to GSK923295A is both high yielding and robust, and the resulting process has been demonstrated first on the kilo scale and subsequently in the pilot plant where 55 kg was successfully prepared. The resulting process is simple, uses cheaper raw materials, is greener in that it avoids using aluminum, tin, and bromination chemistries, and obviates the need for chromatographic purification. Also discussed are the route derived impurities, how they were unambiguously prepared to confirm structure and processing amendments to control their formation, and enhancements to the new process to facilitate future processing.

Introduction

Kinesins are a superfamily of motor proteins that use the energy from ATP hydrolysis to transport cellular cargoes along microtubules.[1] Mitotic kinesins are a functional class of kinesins essential for mitotic spindle assembly and function during cell division.[2] Perturbation of mitotic kinesin activity results in cell cycle arrest in mitosis and subsequent cell death. Centromere-associated protein E (CENP-E) is a mitotic kinesin motor protein functioning exclusively in mitosis to integrate mitotic spindle mechanics with mitotic checkpoint signaling. Disruption of CENP-E function using a variety of methods, including small molecule inhibition of CENP-E kinesin motor domain function, causes cell cycle arrest in mitosis with a bipolar mitotic spindle with misaligned chromosomes, often followed by cell death.[3] GSK923295A is a novel and selective inhibitor of CENP-E motor domain enzymatic activity and is currently in phase I clinical trials for the treatment of cancer.

Herein is described the limitations of the original supply route, the new route investigation and subsequent scale-up, as well as identification and control of some of the key route impurities.

Results and Discussion

Initial Supply Route to GSK923295A. The existing route used in outsource campaigns was 10 stages long and delivered GSK923295A in an overall yield of 4.1% (Scheme 1). The chemistry involved chemoselective reduction of the expensive bromophenylalanine **1** to the corresponding alaninol **2** using lithium aluminum hydride, where careful temperature control was needed to avoid accompanying reduction of the key bromide function. The subsequent Mitsunobu coupling of **2** with phthalimide to give **3** and Stille coupling with the expensive tin reagent **4** to yield the vinyl ether **5** proceeded with the concomitant formation of nonideal tin waste (4 kg tin waste per kg of API). Bromination of **5** provides the halide **6**, which condenses with aminopyridine **7**[4] to secure the benzimidazole **8**. Acid-mediated removal of the Boc function in **8** and acylation with **9**[5] led to the amide **10**. Removal of the phthalimide nucleus with hydrazine and acylation with dimethyl glycine provided GSK923295A after chromatography and crystallization. Overall, the initial supply route consistently failed to deliver sufficient active pharmaceutical ingredient (API) to adequately fund ongoing clinical studies.

Identification of a New Route to GSK923295A 1 via Phthalimide 11. To address the aforementioned issues, we began to explore alternative approaches for the synthesis of the key phenacyl halide functionality. We postulated that such a functionality could be incorporated using Friedel−Crafts chemistry,[6] and evaluation of the literature showed that the amino phthalimide **11**, accessible from L-phenylalaninol **12**, was a known compound[7] that could serve as a substrate for just such

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(1) (a) Vale, R. D. *Cell* **2003**, *112*, 467–480. (b) Miki, H.; Setou, M.; Kaneshiro, K.; Hirokawa, N. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 7004–7011. (c) Goldstein, L. S.; Philip, A. V. *Ann. Rev. Cell Dev. Biol.* **1999**, *15*, 141.

(2) (a) Bergnes, G.; Brejc, K.; Belmont, L. *Curr. Top. Med. Chem.* **2005**, *5*, 127. (b) Sharp, D. J.; Rogers, G. C.; Scholey, J. M. *Nature* **2000**, *407*, 41.

(3) Wood, K. W.; Cornwell, W. D.; Jackson, J. R. *Curr. Opin. Pharmacol.* **2001**, *1*, 370.

(4) The aminopyridine **7** was provided by Dr Reddy's and was derived from an asymmetric reduction on the corresponding 2-acetyl-2-aminopyridine.

(5) The acid chloride **9** was commercially available from Minakem but can also be easily accessed from the readily available ethyl 4-hydroxybenzoate in four stages. Please see Supporting Information.

(6) Miyahara, Y. *J. Heterocycl. Chem.* **1979**, *16*, 1147.

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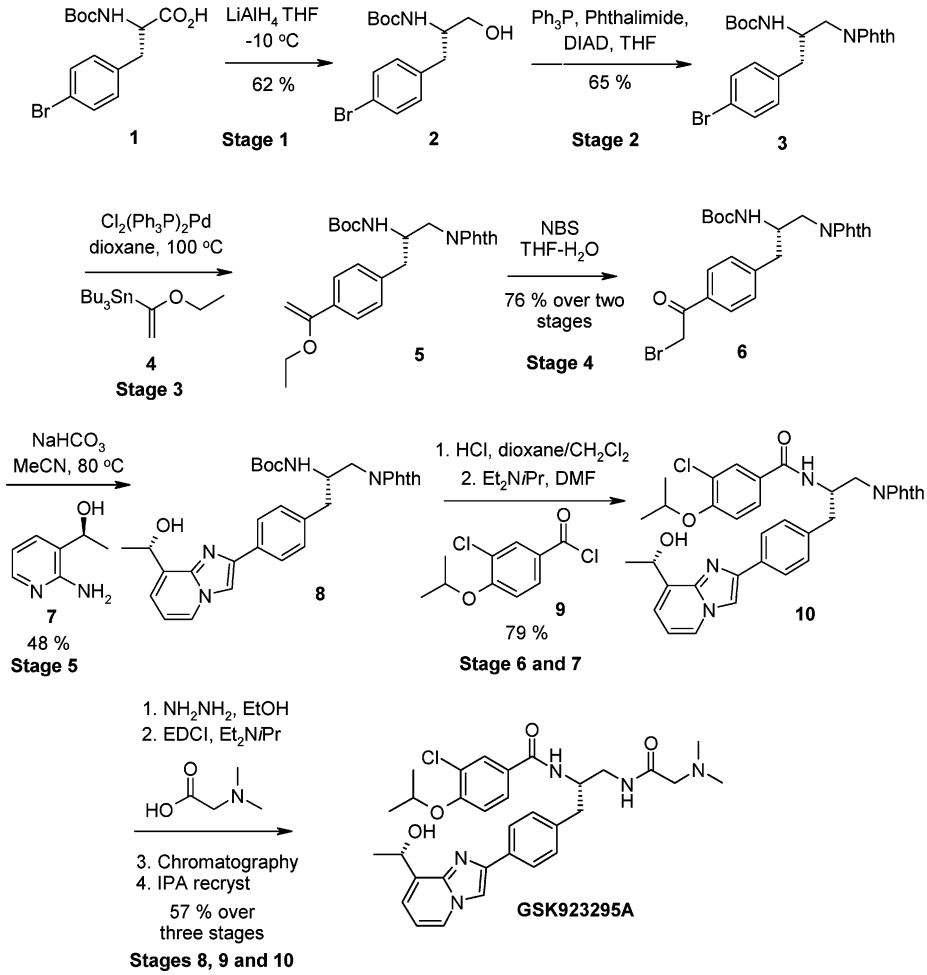
Scheme 1. **Initial supply route to GSK923295A**



an alternative approach to GSK923295A via the phthalimide intermediate **11** (Figure 1).

A further benefit of this approach was that L-phenylalaninol **12** is readily available since it is derived from a natural amino acid. The synthesis of the ammonium chloride **11** commenced by protection of the primary amino function within L-phenylalaninol **12** using Boc anhydride to give the intermediate carbamate **13** in excellent yield. Conversion to the methanesulfonate ester **14** was accomplished again in excellent yield, and this compound was smoothly converted through to the differentially protected diamine **15** by reaction with potassium phthalimide. Finally, acid-mediated hydrolysis of **15** gave **11** in effectively quantitative yield (Scheme 2). For expediency, the above chemistry was telescoped such that the hydroxy carbamate **13** was not isolated but was directly converted through to the methanesulfonate ester **14**.

The above route to amino phthalimide **11** suffered from one drawback in that the intermediate methanesulfonate ester **14** is thermally unstable and starts to undergo an *intramolecular* cyclization at elevated temperatures (>45 °C) to cyclic carbamate **16**.[8] This was easily managed by controlling the temperature of formation and most specifically during the isolation and drying processes.

Pleasingly, the ammonium salt **11** underwent an $AlCl_3$-promoted Friedel−Crafts acylation (Scheme 3) with chloroacetyl chloride in excellent conversion and good selectivity in favor of the desired *para* product **17**, and as expected, the amine function was effectively removed from the reaction as the corresponding hydrochloride salt. Interestingly, it was shown that the minor isomer was not the expected *ortho* isomer but rather the *meta* isomer **18**. We postulated that this unusual isomer pattern was due to the *ortho* position being too sterically congested to undergo acylation and that the weak electronic donation from the alkyl system results in the available *meta* position being more electronically favored than originally expected. The electronic deficiency of the aromatic ring is

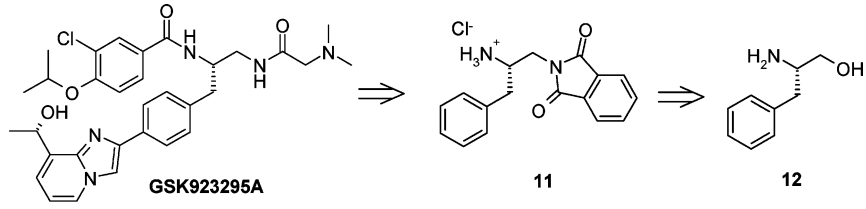
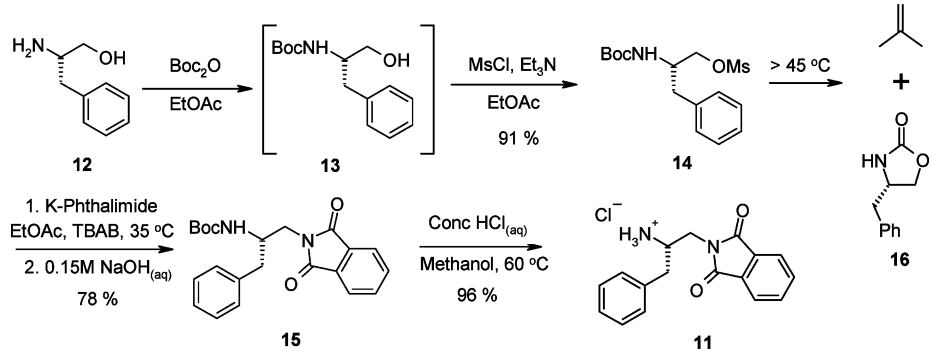
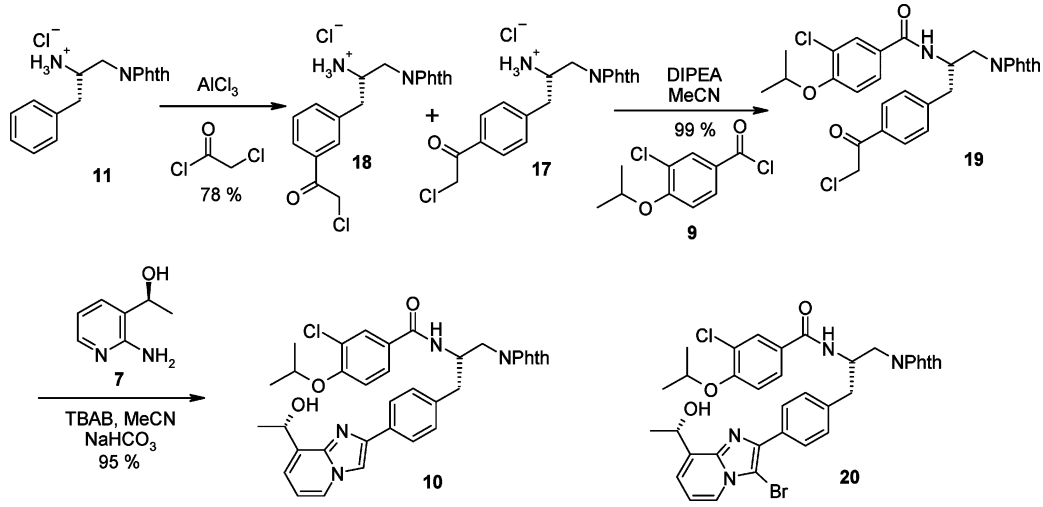


Figure 1. **Retrosynthetic analysis of GSK923295A to L-phenylalaninol 12.**

Scheme 2. **Preparation of amino phthalimide 11 from L-phenylalaninol 12**



Scheme 3. **Friedel−Crafts acylation of 11 and conversion through to phthalimide heterocycle 10**



highlighted further by the limited choice of Lewis acids capable of promoting acylation, and only the group 13 halides of aluminum and gallium were shown to promote acylation to any significant extent. All other Lewis acids, including the reactive lanthanide triflates,[9] showed no reaction or decomposition under forcing conditions. Optimization of the reaction conditions showed that the reaction performed best when conducted in neat chloroacetyl chloride (20 equiv). The selectivity of the reaction improves at lower temperatures, with −5 °C being selected as optimal providing a *para*/*meta* ratio of 4.5/1. No advantage was gained using bromoacetyl bromide with $AlBr_3$.

Quenching the crude reaction mixture into acidic aqueous alcohols and subsequent filtration gave a product where the *para*/*meta* ratio was enhanced with respect to the reaction solution. This key observation demonstrated that the *meta* isomer **18** was more soluble than the desired *para* product **17**. Subsequent development showed the *meta* isomer **18** could be solvated exclusively by heating the quenched reaction mixture to reflux. Subsequent cooling and filtration routinely allowed isolation of the desired *para* product **17** with minimal *meta* contamination (>50/1 *para*/*meta*). Analysis of the filtrate demonstrated the utility of this process showing >80% PAR *meta* product **18**.[10]

The conversion of the phenacyl chloride **17** through to benzamide **19** was highly chemoselective and resulted in a very clean and high yielding reaction. No impurities resulting from reaction of the primary amine of **17** with the phenacyl chloride functionalities in either **17** or **19** were observed. Reaction of the phenacyl chloride **19** with the aminopyridine **7** proved to be a slow transformation. The rate for this reaction could be markedly enhanced by the addition of the phase transfer catalyst tetrabutylammonium bromide (TBAB). It was postulated that this rate enhancement was attributable to the conversion of the phenacyl chloride to the corresponding bromide, and this is confirmed by the formation of an intermediate during this reaction as visible by HPLC. An interesting byproduct was formed during this chemistry at low levels (up to 0.5% PAR) and was confirmed to be common to both the early supply route and the new route. LCMS confirmed the structure as the brominated pyridyl imidazole **20**, and this was confirmed unambiguously through synthesis. Treatment of imidazole **10** with *N*-bromo succinimide (NBS) in DMF resulted in an instantaneous conversion to the corresponding brominated analogue **20** in quantitative yield. This reactivity of pyridyl

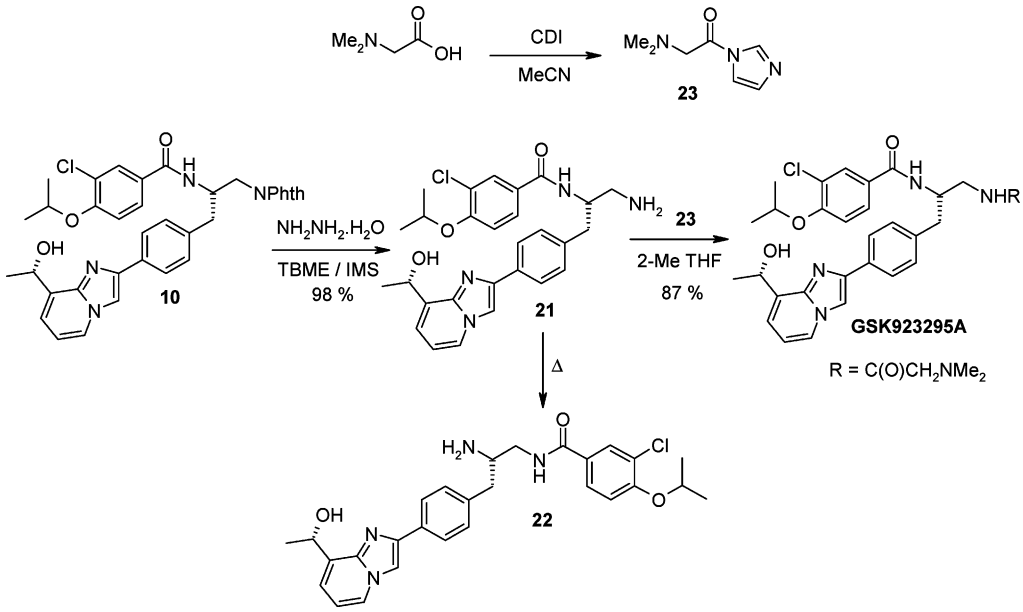
(7) Karavoltsos, M.; Mourtas, S.; Gatos, D.; Barlos, K. *J. Pept. Sci.* **2002**, *8*, 615.

(8) See supporting information for DSC, TGA and ARC thermal safety data.

(9) (a) Dzudza, A.; Marks, T. J. *J. Org. Chem.* **2008**, *73*, 4004. (b) Repichet, S.; Le Roux, C.; Dubac, J.; Desmurs, J.-R. *Eur. J. Org. Chem.* **1998**, 2743.

(10) Protecting the primary amine function as an amide prior to the Friedel Crafts chemistry was evaluated, and the benzoyl and trifluoroacetamide groups showed particular promise. Although slightly higher selectivities for the *para* acylation were observed with these substrates, the requirement for a protection and deprotection strategy made this approach less desirable than simply using the ammonium salt.

Scheme 4. **Conversion of phthalimide 10 through to GSK923295A**



imidazoles toward NBS is known,[11] but the exact mechanism for oxidation of the bromide ion to a source of electrophilic bromine within the reaction medium is currently unknown.

The phthalimide **10** was smoothly converted to the amine **21** using hydrazine hydrate in denatured ethanol. It was important to leave this reaction for an extended period of time to ensure all of the initially formed *N*-aminophthalimide rearranges to the more thermodynamically stable phthaloylhydrazide,[12] thereby rendering the reaction irreversible. It was observed that minor nonbasic impurities could readily be removed from this product by incorporating an extractive workup of **21** into aqueous lactic acid and subsequent basification of the aqueous phase, extraction, and isolation to give **21** with a typical purity of 98% PAR. Temperature control of these reaction conditions was needed to minimize a back ground "trans-amidation" process whereby **21** could rearrange to the isomeric secondary amine **22**. Under the reaction conditions used, the level of **22** was always maintained below 0.2% PAR. To improve the efficiency of the route further, a higher yielding coupling process was sought for the final stage conversion of amine **21** to GSK923295A. Experience of activating the acid as the imidazole ester for amide synthesis has been gained "in-house", and this approach has also been successfully used within the literature with dimethyl glycine.[13] Following this precedent, the preparation of GSK923295A was accomplished in excellent yield by first preparing an imidazole ester of dimethyl glycine **23** by reaction with carbonyl diimidazole (CDI), and adding 1 equiv of this reagent to amine **21** (Scheme 4). Recrystallization of GSK923295A from propan-2-ol delivered clinical grade material in 85% yield.

This route to GSK923295A was scaled up to the 20 L level where approximately 1 kg of API was prepared in two batches and was of excellent purity.

During the release analysis of GSK923295A, one new impurity was identified which was present at the qualification limit and had the same HPLC retention time as the known acetamide impurity **24**. Structural elucidation of this new impurity was paramount in order to facilitate the release process. LCMS suggested and accurate mass highlighted a likely molecular formula ($C_{32}H_{34}ClN_5O_5$). It had also been observed that this impurity had increased during the azeotropic drying process of the API solution prior to addition of antisolvent. A tentative structure **25** was proposed based on the possible presence of sarcosine within the commercial dimethyl glycine which would form via the intermediate **26** (Scheme 5). The structure of this impurity was confirmed through synthesis.[14] Analysis confirmed hydantoin **25** as the new impurity, and retrospective ^{1}H NMR analysis of the dimethyl glycine used within the scale-up campaign showed that levels of sarcosine were indeed present at 0.3%, and the level of sarcosine within dimethyl glycine would need to be controlled through appropriate specification.

As a consequence of the hazardous nature of hydrazine hydrate and particularly with a view to further scale-up, alternative conditions were screened to remove the phthalimide nucleus. Although methylamine[15] and ethylene diamine[16] were identified as potential alternatives for this reaction, the quality of product **21** derived from these conditions was less pure than that from the hydrazine process. It was likely that further development would have led to either of these procedures being suitable for replacing hydrazine hydrate; however, with the tight delivery timelines for the project and implementation of suitable engineering within the pilot plant, it was decided to continue using hydrazine hydrate for this transformation.

(11) (a) Salgado-Zamora, H.; Velazquez, M.; Mejia, D.; Campos-Aldrete, M. E.; Jimenez, R.; Cervantes, H. *Heterocycl. Commun.* **2008**, *14*, 27. (b) Godovikova, S. N.; Gol'dfarb, Ya. L. *Bulletin of the Academy of Sciences of the USSR, Division of Chemical Science (English Translation)* **1965**, 1391.

(12) (a) Drew, D. K.; Hatt, H. H., J. Chem. Soc., 1937, Abstracts: 16. (b) Chattaway, F. D.; Tesh, W. *J. Chem. Soc.* **1920**, *117*, 711. (c) Hearn, M. J.; Prisch, S. B. *Org. Prep. Proc. Int.* **1981**, *87*, 421.

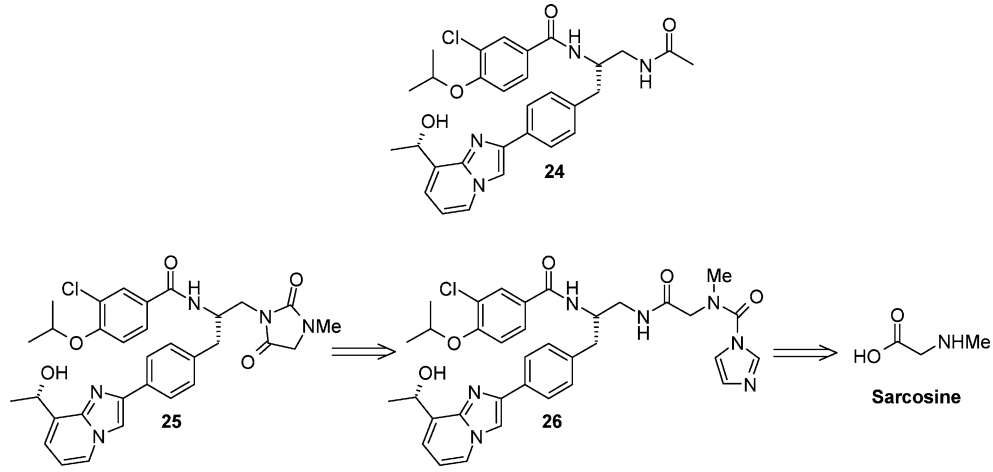
(13) (a) Gerecke, M.; Kyburz, E.; Borer, R.; Gassner, W. *Heterocycles* **1994**, *39*, 693. (b) Johnson, D. W. *J. Mass Spectrom.* **2001**, *36*, 277.

(14) See supporting information for details.

(15) (a) Khan, M. N.; Ohayagha, J. E. *React. Kinet. Catal. L.* **1996**, *58*, 97.

(16) Bauer, J.; Rademann, J. *Tetrahedron Lett.* **2003**, *44*, 5019.

Scheme 5. **Elucidation and synthesis of the hydantoin impurity 24**



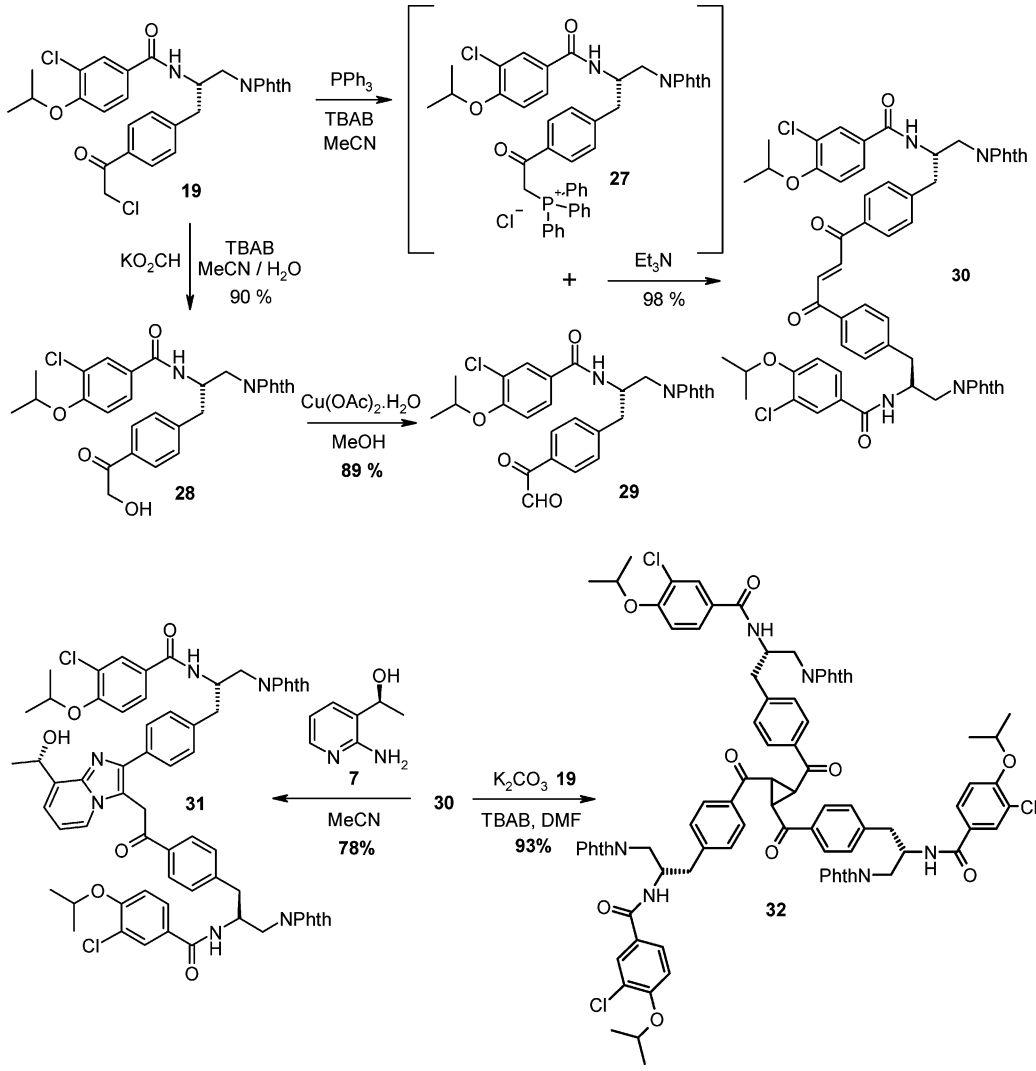
The process to GSK923295A was transferred to pilot plant, and a total of 55 kg was prepared. On increased scale, however, the reaction of phenacyl chloride **19** with aminopyridine **7** resulted in very impure product **10** (85% assay) as a consequence of the semibatch addition mode used. The process involved stirring the phenacyl chloride **19** with sodium bicarbonate in acetonitrile, heating to the reaction temperature, and subsequently adding a solution of the aminopyridine **7**. Under these conditions, the phenacyl chloride **19** proves to be unstable and undergoes base-mediated self-condensation, and this was exacerbated on plant by the prolonged heating and addition times. Two of the main byproducts were identified by spectroscopy as diamide **31** and cyclopropane **32**, and their structures were unambiguously confirmed through synthesis (Scheme 6). This reactivity of phenacyl halides to base is well-known,[17] and this undesired reactivity is easily remedied by returning to a formal batch process. The incorporation of an acetonitrile slurry on the pyridyl imidazole **10** product resulted in a significant purification to 95% assay. The synthesis of the two key impurities **31** and **32** present in this large-scale batch of **10** was required to deliver valuable reference materials. The direct approach of degrading the phenacyl chloride using base led to a multicomponent mixture from which it was difficult to isolate the desired products. A retrosynthetic analysis suggested that these two compounds were probably accessed via the same enedione intermediate **30**, and it was decided to prepare this compound from a Wittig reaction between the carboxaldehyde **29** and the phosphonium salt **27**. The preparation of Wittig reagents from chloroacetyl benzene is known;[18] similarly, reaction of the phenacyl chloride **19** with triphenyl phosphine gave the phosphonium salt **27** in excellent yield. The carboxaldehyde **29** was also accessed from the phenacyl chloride **19** in two steps. Initial attempts to hydrolyze **19** led to significant byproducts forming through aldol-type condensations, or oxidations, of the product **28**, which proved a somewhat sensitive entity. We developed a method for the smooth and particularly mild conversion of **19** to the hydroxy ketone **28** using potassium formate to form an intermediate formate ester. Ester hydrolysis was achieved in situ under the aqueous conditions used for the reaction to give the hydroxy ketone **28** in excellent yield. The process was conducted under nitrogen to prevent undesired oxidative byproducts. There are reports in the literature whereby phenacyl chlorides can be converted to the corresponding hydroxy ketones using sodium[19] or potassium acetate,[20] but in our hands, the reactions were less clean, required acid hydrolysis, and consequently led to the undesired aldol chemistry unless very carefully controlled. Oxidation of the hydroxy ketone to the glyoxal **29** was accomplished in excellent yield using copper acetate.[21] Workup for this reaction proved critical, and filtration of inorganic residues followed by removal of solvent prior to extraction proved necessary to minimize polymer formation. Wittig reaction of the phosphonium salt **27** with glyoxal **29** using triethylamine as base[22] gave the target diketone **30** in near quantitative yield. Condensation of diketone **30** with the aminopyridine **7** led to the ketone **31** in very good yield, and in addition, the base-mediated reaction of enedione **30** with phenacyl chloride **19** gave the cyclopropane **32**, also in excellent yield. Both ketone **31** and cyclopropane **32** were confirmed to be the impurities present within the crude pyridyl imidazole **10**.

Following the successful pilot plant campaign, attention was focused on some of the processing concerns that would prove troublesome for future scale-up campaigns. These concerns included: (1) The methanesulfonate ester **14** was shown to be mutagenic and was isolated in the current process. (2) Acetonitrile is a class A volatile organic compound and was used in two stages to prepare compounds **19** and **10**. (3) Aminopyridine **7** is a highly viscous oil that required solvation to handle. (4) The coupling of amine **21** with the imidazole ester **23** requires titration of the two reagents.

To address the occupational hygiene concerns of isolating the mutagenic methanesulfonate **14**, an alternative

(17) (a) Saba, A. *J. Chem. Res.* **1990**, *9*, 2227. (b) Pasto, D. J.; Garves, K.; Sevenair, J. P. *J. Org. Chem.* **1968**, *33*, 2975.
(18) Uziel, J.; Riegel, N.; Aka, S.; Figuiere, P.; Juge, S. *Tetrahedron Lett.* **1997**, *38*, 3405.
(19) Miyauchi, H.; Nakamura, T.; Ohashi, N. *Bull. Chem. Soc. Jpn.* **1996**, *69*, 2625.
(20) Tozuka, H.; Ota, M.; Kofujita, H.; Takahashi, K. *J.Wood Sci.* **2005**, *51*, 48.
(21) (a) Nef, *Justus Liebigs Ann. Chem.* **1904**, *335*, 82. (b) Evans, *American Chemical Journal* **1906**, *35*, 124.
(22) Konno, T.; Takehana, T.; Mishima, M.; Ishihara, T. *J. Org. Chem.,* **2006**, *71*, 3545.

Scheme 6. **Preparation of impurities derived from the synthesis of 10**



route to the amino phthalimide **11** was investigated from L-phenylalaninol **12**. Activation of the alcohol **13** in situ utilizing the well-recognized Mitsunobu approach[23] presented an alternative and potentially simpler one-pot procedure toward alkylation. Gratifyingly, the DIAD/PPh_3-mediated displacement with phthalimide readily provided the desired **15**. There was no competing *intramolecular* cyclization, and the need for a caustic slurry to remove undesired phthalimide was obviated, thereby saving on processing time. Moreover, the generated triphenylphosphine oxide could be efficiently solubilized by addition of alcoholic solvents while also acting as an antisolvent for the desired product, thereby providing a simplified workup procedure. This protocol was easily incorporated with the *tert*-butyl carbamate protection and when performed in toluene allowed for removal of the *tert*-butyl alcohol byproduct through distillation that would otherwise consume an equivalent of DIAD and triphenylphosphine by dehydration. A further benefit of this strategy was that *tert*-butyl carbamate cleavage could also be performed in the same reaction by the addition of methanol/conc $HCl_{(aq)}$, thus providing a simple three-stage one-pot telescoped procedure for the synthesis of **11** in 86% yield over the three steps (Scheme 7).

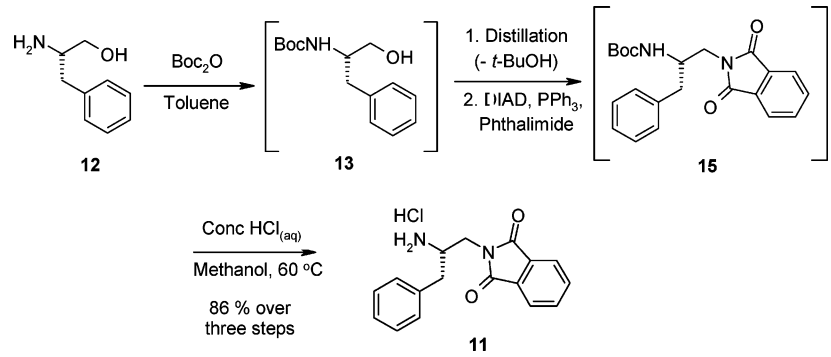
To avoid the use of acetonitrile, a solvent screen was conducted for the preparation of both intermediates **10** and **19**. It was quickly demonstrated that THF was a good alternative with comparable yields of product being obtained in both cases.

Handling concerns associated with the use of the aminopyridine **7** were readily addressed by preparing the corresponding hydrochloride salt **33**, which proved to be a highly granular and free-flowing solid. The preparation was facile and very high yielding and involved treating an ethyl acetate solution of **7** with 4−6 N hydrochloric acid in propan-2-ol. This material performed very well in the reaction to prepare pyridyl imidazole **10** from phenacyl chloride **19** and simply required the addition of an extra equivalent of base.

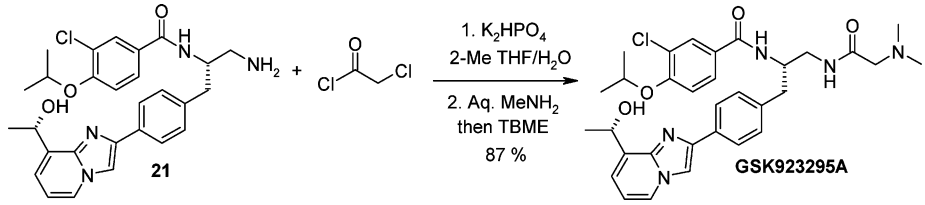
Finally, the procedure to prepare GSK923295A had required effective titration of the imidazole ester **23** with the amine **21** to avoid undesired secondary O-acylation. This had not proved an issue in the pilot plant but did require an element of control, and as such, it was desirable to avoid complicated processing. Work to prepare the hydantoin impurity **25** from amine **21** (Scheme 5) had demonstrated that reaction of **21** with chloro-

(23) Kumara Swamy, K. C.; Bhuvan Kumar, N. N.; Balaraman, E.; Pavan Kumar, K. V. P. *Chem. Rev.* **2009**, *109*, 2551.

Scheme 7. **Telescoped conversion of alcohol 12 through to amino phthalimide 11 using a Mitsunobu approach**



Scheme 8. **Modified Schotten-Baumann conditions for the preparation of GSK923295A**



acetyl chloride leads to an intermediate chloroacetamide that can react with amines. Extension of this chemistry by quenching with dimethylamine did deliver GSK923295A, but the byproducts derived from overacylation were still apparent at low levels. To circumvent this overacylation, Schotten-Baumann chemistry was developed, where by reaction of **21** with chloroacetyl chloride under the biphasic conditions of water and 2-methyltetrahydrofuran, using an appropriate buffer, led to a highly N-selective acylation.[24] Subsequent treatment with aqueous dimethylamine led to an excellent conversion to GSK923295A, delivering a simpler and more robust process that would be used in any future campaigns (Scheme 8).

Summary

To conclude, a robust and scalable route to GSK923295A has been discovered and developed which is both shorter and higher yielding than the original supply route (33.6 versus 4.1%). The route exemplifies highly chemoselective Friedel–Crafts chemistry, which obviates the need for both costly brominated starting materials and nongreen tin chemistry. An excellent understanding of the main route derived impurities has also been demonstrated through their unambiguous synthesis, which in itself demonstrates some interesting and versatile synthetic methods but also leads to a greater understanding of the chemical process. Additionally, the route has been further developed to design out complexity such that future campaigns will have less isolations, avoid handling of mutagenic materials, and have inherently more robust chemistry. It is anticipated that the overall yield will increase to 38% for the fully developed process.

Experimental Section

General. Commercially available reagents were used without further purification. Reactions requiring anhydrous conditions were performed under an atmosphere of dry nitrogen. 4-Isopropoxy-3-chloro benzoyl chloride and 2-amino-3-(1*S*)-1-hydroxyethylpyridine were obtained commercially from custom synthesis suppliers. The experimental conditions below refer to the procedures used for the multikilogram pilot plant campaign. For a key to ^{1}H NMR ring assignments, see Supporting Information.

(2*S*)-2-({[(1,1-Dimethylethyl)oxy]carbonyl}amino)-3-phenylpropyl methanesulfonate (14): L-Phenylalaninol **12** (500 g, 3.31 mol) was suspended in ethyl acetate (6.67 L) and treated with di(*tert*)-butyl dicarbonate (794 g, 3.64 mol). On complete reaction (1.5 h), the reaction mixture was cooled to 0 °C and treated with triethylamine (608 mL, 4.37 mol). Methanesulfonyl chloride (281 mL, 414 g, 3.64 mol) was added dropwise, maintaining an internal temperature of below 4 °C at all times. Water (4.17 L) was added, and the ethyl acetate was removed by distillation under vacuum. The product was collected by filtration under vacuum, washed with water (4.0 L), and dried under vacuum to yield the title compound (1.05 kg, 95.9%) as a colorless solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 1.40 (9H, s, $(CH_3)_3$), 2.69–2.95 (2H, m, CH_2Ph), 3.01 (3H, s, SO_2CH_3), 4.05–4.13 (2H, m, CH_2O), 4.20–4.29 (1H, m, C*H*NH), 4.73 (1H, br d, CHN*H*), 7.18–7.38 (5H, m, C*H* Ar); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 28.3, 37.3, 50.9, 69.9, 80.0, 127.0, 128.8, 129.3, 136.7 and 155.2; HRMS (ESI$^+$) *m/z* calcd for $[M + Na]^+$ $C_{15}H_{23}NO_5SNa$ 352.1195, found 352.1182.

1,1-Dimethylethyl [(1*S*)-2-(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)-1-(phenylmethyl)ethyl]carbamate (15): Potassium phthalimide (646.3 g, 3.49 mol) was combined with (2*S*)-2-({[(1,1-dimethylethyl)oxy]carbonyl}amino)-3-phenylpropyl methanesulfonate **14** (1.05 kg, 3.17 mol), tetrabutylammonium bromide (133 g, 0.41 mol), and isopropyl acetate (8.36 L). This mixture was warmed to 35 °C and stirred for 23 h. Isohexane (8.36 L) was added, and the reaction mixture was cooled to 0 °C. The crude product was isolated by filtration, washed with a mixture of isohexane and isopropyl acetate (1:1, 3 L), and dried under vacuum. The crude solid was suspended in 0.15 M NaOH (10.0 L) and stirred at room temperature for 1 h. The

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mixture was isolated by filtration and the cake washed with water (3 L). The product was dried under vacuum to yield the title compound (1.05 kg, 87.1%) as a colorless solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 1.22 (9H, s, $(CH_3)_3$), 2.72−2.95 (2H, m, CH_2Ph), 3.62−3.79 (2H, m, CH_2NPhthal), 4.20−4.35 (1H, m, C*H*NH), 4.64 (1H, br d, CHN*H*), 7.18−7.38, 7.65−7.73 and 7.80−7.88 (9H, m, C*H* Ar); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 28.1, 39.1, 41.8, 50.3, 79.3, 123.3, 126.7, 128.6, 129.2, 132.0, 133.9, 136.9, 155.5 and 168.5; HRMS (ESI$^+$) *m*/*z* calcd for $[M + Na]^+$ $C_{22}H_{24}N_2O_4Na$ 403.1634, found 403.1615.

2-[(2*S*)-2-Amino-3-phenylpropyl]-1*H*-isoindole-1,3(2*H*)-dione hydrochloride (11). *Pilot Plant Procedure:* To a warmed (60 °C) suspension of 1,1-dimethylethyl[(1*S*)-2-(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)-1-(phenylmethyl)ethyl]carbamate **15** (1.05 kg, 2.76 mol) in methanol (10.5 L) was added concentrated hydrochloric acid (1.22 L, 1.44 kg, 14.64 mol) over 1 h (**caution: gas evolution**). The resulting slurry was stirred at 60 °C for 2.25 h. The mixture was cooled to 0 °C, the slurry isolated by filtration, and the cake washed with propan-2-ol (2.4 L). The solid was dried under vacuum to yield the title compound (0.786 kg, 89.8%) as a colorless solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ_H 2.86 (1H, dd, J = 14.0 and 4.0, CH_2^aPh), 3.20 (1H, dd, J = 14.0 and 4.0, CH_2^bPh), 3.64 (1H, dd, J =13.5 and 4.0, CH_2^aNPhthal), 3.74 (1H, m, C*H*NH), 3.75 (1H, dd, J =13.5 and 4.0, CH_2^bNPhthal), 3.62−3.79 (2H, m, CH_2NPhthal), 7.18−7.28, 7.30−7.35 and 7.80−7.88 (9H, 3 × m, C*H* Ar), 8.41 (3H, br s, NH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ_C 36.5, 39.0, 50.3, 122.9, 126.7, 128.5, 129.0, 131.7, 134.2, 136.2 and 168.0; HRMS (ESI$^+$) *m*/*z* calcd for $[M - Cl]^+$ $C_{17}H_{17}N_2O_2$ 281.1290, found 281.1288.

*Optimized Telescoped Procedure to Compound **11**:* L-Phenylalaninol **12** (15.0 g, 0.099 mol) was suspended in toluene (90 mL) with mechanical stirring, and the mixture was treated with di-*tert*-butyl dicarbonate (22.7 g, 0.104 mol). The reaction mixture was warmed to 40 °C and stirred until all solids dissolved (1.5 h). The reaction mixture was heated and distillation initiated and continued until all *tert*-butyl alcohol was removed as determined by ^{1}H NMR analysis. The reaction mixture was cooled to 70 °C, diluted with toluene (141 mL), and further cooled to 42 °C. The reaction mixture was treated with triphenyl phosphine (35.52 g, 0.135 mol) and phthalimide (15.30 g, 0.104 mol), and DIAD (24.6 mL, 25.1 g, 0.124 mol) was added, maintaining a temperature between 42 and 52 °C. After 10 min, methanol (350 mL) was added and the reaction warmed to 60 °C. Concentrated hydrochloric acid (35 mL) was added dropwise over 1 h, and the resulting suspension was stirred for a further hour before cooling to 5 °C (**caution: gas evolution**). The product was isolated by filtration and the cake washed with propan-2-ol (45 mL). The solid was dried under vacuum to yield the title compound (27.1 g, 86%) as a colorless solid that was spectroscopically identical to that reported above.

2-{(2*S*)-2-Amino-3-[4-(chloroacetyl)phenyl]propyl}-1*H*-isoindole-1,3(2*H*)-dione (17): 2-[(2*S*)-2-Amino-3-phenylpropyl]-1*H*-isoindole-1,3(2*H*)-dione hydrochloride **11** (0.785 kg, 2.48 mol) was suspended in chloroacetyl chloride (3.95 L, 5.6 kg, 49.6 mol) and cooled to −5 °C with mechanical stirring. This suspension was then treated with aluminum chloride (1.65 kg, 12.4 mol). The resulting mixture was stirred at −5 °C for 22 h, after which the reaction mixture was carefully added to methanol (15.7 L), maintaining a temperature below 35 °C. The resulting stirred mixture was warmed to 50 °C and treated with 3.5 M hydrochloric acid (3.9 L). The mixture was heated further to 65 °C, stirred at this temperature for 18 h, then cooled to ambient. The crude product was collected by filtration and washed with methanolic hydrogen chloride comprising methanol (2.35 L) and 3.5 M hydrochloric acid (0.785 L). The crude product was purified further by suspending in a mixture of methanol (7.85 L) and 3.5 M hydrochloric acid (3.14 L) and warmed to 65 °C. The product was then isolated by filtration, washed with methanol (1.57 L), and dried under vacuum to yield the title compound (0.703 kg, 72.1%) as a colorless solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ_H 2.98 (1H, dd, J = 14.0 and 9.0, CH_2^aPh), 3.28 (1H, dd, J = 14.0 and 4.0, CH_2^bPh), 3.67 (1H, dd, J =13.3 and 4.0, CH_2^aNPhthal), 3.74−3.89 (2H, m, C*H*NH and CH_2^bNPhthal), 5.15 (2H, s, CH_2Cl), 7.52 (2H, d, J = 8.0, CH Ar Phthal), 7.81 (5H, m, C*H* Ar), 7.92 (2H, d, J = 8.0, CH Ar Phthal), 8.46 (3H, br s, NH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ_C 36.4, 39.0, 47.6, 50.3, 122.9, 128.6, 129.5, 131.8, 132.8, 134.2, 142.6, 167.9 and 191.1; HRMS (ESI$^+$) *m*/*z* calcd for $[M - Cl]^+$ $C_{19}H_{18}N_2O_3$ 357.1006, found 357.0992.

Data for *meta* isomer 2-{(2*S*)-2-amino-3-[2-(chloroacetyl)-phenyl]propyl}-1*H*-isoindole-1,3(2*H*)-dione (**18**): ^{1}H NMR (400 MHz, DMSO-d_6) δ_H 3.02 (1H, dd, J = 14.0 and 8.5, CH_2^aPh), 3.30 (1H, dd, J = 14.0 and 4.5, CH_2^bPh), 3.72 (1H, dd, J =14.0 and 4.5, CH_2^aNPhthal), 3.74−3.95 (2H, m, C*H*NH and CH_2^bNPhthal), 5.20 (2H, d, J = 3.0, CH_2Cl), 7.50 (1H, dd, J = 7.5 and 7.5, CH Ar Phthal), 7.68 (1H, d, J = 7.5, C*H* Ar), 7.80 (5H, m, CH Ar), 7.95 (1H, s, C*H* Ar), 8.60 (3H, br s, NH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ_C 36.1, 39.0, 47.8, 50.2, 122.9, 126.9, 129.0, 129.1, 131.8, 134.2, 134.5, 134.6, 137.0, 168.0 and 191.4; HRMS (ESI$^+$) *m*/*z* calcd for $[M - Cl]^+$ $C_{19}H_{18}N_2O_3$ 357.1006, found 357.0993.

3-Chloro-*N*-{(1*S*)-2-[4-(chloroacetyl)phenyl]-1-[(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)methyl]ethyl}-4-[(1-methylethyl)oxy]benzamide (19): 2-{(2*S*)-2-Amino-3-[4-(chloroacetyl)phenyl]propyl}-1*H*-isoindole-1,3(2*H*)-dione **17** (0.70 kg, 1.78 mol) was suspended in acetonitrile (9.1 L) and cooled to 5 °C with stirring. Diisopropylethylamine (0.78 L, 0.575 kg, 4.45 mol) was added dropwise to the stirred mixture followed by a solution of 3-chloro-4-[(1-methylethyl)oxy]benzoyl chloride **9** (0.441 kg, 1.89 mol) in acetonitrile (1.3 L), maintaining an addition temperature below 8 °C. The mixture was stirred at 5−8 °C for 1 h. Water (10.5 L) was added and the mixture stirred at 5 °C for 30 min. The product was isolated by filtration washing with a mixture of acetonitrile (1.75 L) and water (3.0 L). The product was dried under vacuum to yield the title compound (0.962 kg, 97.6%) as a colorless solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 1.42 (6H, d, J = 6.0, $(CH_3)_2$), 3.05 (1H, dd, J = 14.0 and 7.0, CH_2^aPh), 3.15 (1H, dd, J = 14.0 and 7.0, CH_2^bPh), 3.90 (2H, m, CH_2NPhthal), 4.60−4.75 (4H, m, C*H*NH, $(CH_3)_2CHO$ and CH_2Cl), 6.83 (1H, d, J = 7.7, N*H*), 6.96 (1H, d, J = 7.5, *H*-b), 7.42 (2H, d, J = 8.0, *H*-d), 7.58 (1H, dd, J = 7.5 and 2.0, *H*-c), 7.70−7.79 (3H, m, *H*-b and *H*-g), 7.82−7.88 (2H, m, *H*-f), 7.93 (2H, d, J = 8.0, *H*-e); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 21.9, 38.8, 41.0, 46.1, 50.8, 71.9, 114.1, 123.5, 123.8, 126.6, 126.7, 128.8, 129.5, 129.7, 131.7, 132.8, 134.3, 144.0,

156.3, 165.9, 168.8 and 190.7; HRMS (ESI^+) *m/z* calcd for $[M + H]^+$ $C_{29}H_{27}N_2O_5Cl_2$ 553.1297, found 553.1284.

2-Amino-3-[(1*S*)-1-hydroxyethyl]pyridinium chloride (33): (1*S*)-1-(2-Amino-3-pyridinyl)ethanol **7** (25 g, 0.181 mol) was dissolved in ethyl acetate (200 mL) and added dropwise to a cooled stirred 4−6 N solution of hydrogen chloride in propan-2-ol (50 mL, 0.2 mol) over 20 min, maintaining an addition temperature of −5 to 2 °C at all times. The resulting suspension was stirred at <5 °C for 30 min, and the resulting solid was collected by filtration, washed with cold ethyl acetate (2 × 25 mL), and pulled dry to give the title compound as a cream solid (29.9 g, 94.6%): ^{1}H NMR (400 MHz, DMSO-d_6) δ_H 1.32 (3H, d, J = 6.5, CH_3), 4.88 (1H, q, J = 6.5, CHCH$_3$), 5.72 (1H, br s, OH), 6.93 (1H, dd, J = 7.0 and 6.5, CHAr), 7.90−8.00 (4H, m, 2 × CHAr and NH$_2$), 14.18 (1H, br s, ArNHCl);^{13}C NMR (100 MHz, $CDCl_3$) δ_C 22.5, 63.9, 112.3, 130.4, 133.9, 139.3, and 151.8; HRMS (ESI^+) *m/z* calcd for $[M - Cl]^+$ $C_7H_{11}N_2O$ 139.0871, found 139.0867.

3-Chloro-*N*-{(1*S*)-2-(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)-1-[(4-{8-[(1*S*)-1-hydroxyethyl]imidazo[1,2-*a*]pyridin-2-yl}phenyl)methyl]ethyl}-4-[(1-methylethyl)oxy]benzamide (10): Sodium hydrogen carbonate (211.4 g, 2.52 mol), 3-chloro-*N*-{(1*S*)-2-[4-(chloroacetyl)phenyl]-1-[(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)methyl]ethyl}-4-[(1-methylethyl)oxy]benzamide **19** (0.961 kg, 1.74 mol), and tetrabutylammonium bromide (95.14 g, 0.295 mol) were suspended in acetonitrile (10 L) and stirred mechanically. A solution of (1*S*)-1-(2-amino-3-pyridinyl)ethanol **7** (0.258 kg, 1.87 mol) in acetonitrile (1.53 L) was added to the stirred mixture and the suspension heated to reflux. The mixture was stirred at reflux for 26 h. The mixture was cooled to 50 °C and treated with water (12.5 L) before cooling to room temperature. The product was isolated by filtration, washed with water (4.8 L), and dried under vacuum to yield the title compound (1.02 kg, 92.6%) as a colorless solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 1.36 (6H, d, J = 6.0, (CH_3)$_2$), 1.72 (3H, d, J = 7.0, CH_3CH), 3.05 (1H, dd, J = 14.0 and 7.0, CH_2Ph), 3.89 (2H, m, CH_2NPhthal), 4.61 (1H, m, (CH$_3$)$_2$CHO), 4.71 (1H, m, CHNH), 5.27 (1H, q, J = 7.0, CHOH), 5.39 (1H, m, NH), 6.61 (1H, d, J = 7.5, OH), 6.75 (1H, t, J = 7.0, *H*-j), 6.91 (1H, d, J = 7.5, *H*-b), 7.02 (1H, d, J = 7.0, *H*-k), 7.34 (1H, d, J = 8.0, *H*-d), 7.54 (1H, dd, J = 7.5 and 2.0, *H*-c), 7.65−7.71 (2H, m, *H*-g), 7.75 (1H, s, *H*-a), 7.80−7.85 (3H, m, *H*-h and *H*-f), 7.90 (2H, m, *H*-e), 8.03 (1H, d, J = 8.0, *H*-i); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 21.9, 22.5, 38.6, 41.1, 50.8, 67.8, 71.9, 108.0, 112.4, 114.1, 119.7, 123.3, 123.8, 124.3, 126.3, 126.6, 127.1, 129.6, 129.8, 131.7, 132.0, 133.7, 134.1, 136.6, 144.2, 144.4, 156.2, 165.9 and 168.7; HRMS (ESI^+) *m/z* calcd for $[M + H]^+$ $C_{36}H_{34}N_4O_5Cl$ 637.2218, found 637.2238.

3-Chloro-*N*-{(1*S*)-2-[(*N*,*N*-dimethylglycyl)amino]-1-[(4-{8-[(1*S*)-1-hydroxyethyl]imidazo[1,2-*a*]pyridin-2-yl}phenyl)-methyl]ethyl}-4-[(1-methylethyl)oxy]benzamide (GSK923295A). *Deprotection Step:* Hydrazine monohydrate (660 mL, 681.5 g, 13.61 mol) was added dropwise to a stirred suspension of 3-chloro-*N*-{(1*S*)-2-(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)-1-[(4-{8-[(1*S*)-1-hydroxyethyl]imidazo[1,2-*a*]pyridin-2-yl}phenyl)methyl]ethyl}-4-[(1-methylethyl)oxy] benzamide **10** (867.4 g, 1.361 mol) in TBME (10.4 L) and IMS-74OP (3.34 L) at 40 °C over 20 min. The reaction mixture is stirred over 16 h at this temperature then quenched at 40 °C with water (4.34 L). The layers were separated, and the organic layer was washed with water (4.34 L) and the product extracted into an aqueous solution of 10% lactic acid (3.30 L). The acidic aqueous layer is washed with TBME (2.6 L) and then basified to pH10 with 4 M NaOH solution (1.22 L) to liberate free amine. The intermediate **21** was then finally extracted into 2-Me THF (3.47 L), washed with brine (3.47 L) and the solution used directly in the next stage.

Pilot-plant Procedure. N,N′-Dimethylamino glycine (190.6 g, 1.84 mol) is suspended in acetonitrile (0.762 L) and treated with carbonyl diimidazole (286.9 g, 1.76 mol) at room temperature. This solution was stirred at room temperature for 30 min and used in the next step after analysis.

Crude **21** (680 g - *assay*), as a solution in 2-Me THF from above was stirred at 22 °C. The solution of acid imidate **23** was added dropwise. On complete addition, the reaction was stirred at room temperature for 30 min before quenching by the addition of water (5.77 L). The layers were separated and the aqueous extracted with a further potion of 2-Me THF (1.5 L). The organic layers were combined and distilled under dean and stark conditions until <4% w/w water was present by Karl Fischer analysis. The solution was finally distilled to 4.7 L total volume, cooled to 50 °C and treated with TBME (9.5 L). The mixture was stirred at 50 °C for 1 h before cooling to 10 °C and stirring for a further 1 h. The product was isolated by filtration washing with of 2-Me THF/TBME (1/2, 3.0 L). The cake was dried under vacuum to yield the title compound (691 g, 87.0%) as a colorless solid. Recystallization from propan-2-ol (13.6 L) secured clinical grade material (86%). ^{1}H NMR (400 MHz, CD_3OD) δ_H 1.34 (6H, d, J = 6.0, (CH_3)$_2$), 1.59 (3H, d, J = 7.0, CH_3CH), 2.21 (6H, s, N(CH_3)$_2$), 2.87−3.01 (4H, m, CH_2Ph and CH_2N(CH$_3$)$_2$), 3.49 (2H, m, CH_2NPhthal), 4.50 (1H, m, CHNH), 4.70 (1H, m, (CH$_3$)$_2$CHO)), 5.49 (1H, q, J = 7.0, CHOH), 6.88 (1H, t, J = 7.0, *H*-j), 7.08 (1H, d, J = 7.5, *H*-b), 7.33−7.37 (3H, m, *H*-k and *H*-d), 7.63 (1H, dd, J = 7.5 and 2.0, *H*-c), 7.78 (1H, s, *H*-a), 7.83 (2H, d, J = 7.0, *H*-e), 8.09 (1H, m, *H*-h), 8.27 (1H, d, J = 8.0, *H*-i); ^{13}C NMR (100 MHz, CD_3OD) δ_C 22.2, 24.1, 39.3, 43.8, 46.1, 53.0, 63.7, 66.2, 73.0, 110.4, 113.8, 115.3, 121.2, 124.5, 126.1, 127.5, 128.4, 128.5, 130.6, 130.7, 133.3, 136.0, 139.4, 145.1, 146.1, 157.6, 168.5 and 173.6; HRMS (ESI^+) *m/z* calculated for $[M+H]^+$ $C_{32}H_{39}N_5O_4Cl$ 592.2691, found 592.2684.

Alternative Procedure. Intermediate **21** (0.0471 mol) as a cooled (10 °C) solution in 2-Me THF (300 mL) and water (300 mL) was treated with dipotassium hydrogen phosphate (16.4 g, 0.0942 mol) followed by chloroacetyl chloride (4.85 mL, 7.18 g, 0.0636 mol) dropwise. The reaction mixture was then treated with an aqueous solution of dimethylamine (40% w/v, 59.6 mL, 0.0471 mol) and heated to 40 °C. After stirring at for 4 h the solution was cooled to room temperature and the layers separated. The organic layer was further washed with water (250 mL) and resulting organic layer dried by dean and stark distillation until <2% w/w water was present as determined by Karl Fischer analysis. The reaction mixture was concentrated by distillation to 7 vols total volume (210 mL) before cooling to 50 °C and treating with TBME (210 mL). The mixture was cooled to 10 °C and stirred for 1 h before isolating the product

by filtration and washing with 2-Me THF/TBME (1/1, 90 mL). The cake was dried under vacuum to yield the title compound (25.37 g, 90.9%) as a colorless solid that was spectroscopically identical to that reported above.

SYNOPSIS TOC

The rapid discovery and scale-up of a new greener and robust route to the CENP-E inhibitor GSK923295A originating from the cheap and widely L-phenylalaninol is discussed. Also discussed are the route derived impurities, how they were unambiguously prepared to confirm structure and processing amendments to control their formation, as well as enhancements to the new process to facilitate future processing.

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Supporting Information Available

Experimental procedures, characterization data and ^{1}H NMR are available for all isolated compounds. This information is available free of charge *via* the Internet at http://pubs.acs.org/.

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