Process Development and GMP Production of a Potent NAE Inhibitor Pevonedistat

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Chemical Development Laboratories, Millennium Pharmaceuticals Company Limited as a clinical candidate for the treatment of cancer.1 The NEDD8-activating enzyme (NAE) inhibitor, has demonstrated in vitro cytotoxic activity against a variety of human malignancies and is currently being developed by Takeda Pharmaceuticals Company Limited as a clinical candidate for the treatment of cancer.1 The five-membered ring, consisting of three chiral centers and an acid/base sensitive terminal sulfamoyl group, presented considerable challenges for the development of a practical and scalable synthesis of pevonedistat.

The original Discovery synthesis toward pevonedistat involved 20+ linear steps and multiple chromatographic purifications (Scheme 1). More than half of the chemical transformations in the synthesis were carried out to construct the chiral five-member ring. Poor efficiency resulted from a low yielding resolution, removal of an extra chiral hydroxyl group, and installation of a ketol protecting group. Furthermore, the late stage terminal sulfamoylation on the primary hydroxyl group was poorly selective and low yielding.2 As such, the Discovery route was not considered amendable for large-scale production. This report describes the development of a practical and scalable synthesis of pevonedistat from 20+ linear steps and multiple chromatographic purifications to 4 (+)-2,3-Dihydro-1H-inden-1-ylamino-7H-pyrrolo[2,3-d]pyrimidin-7-yl)-2-hydroxycyclopentyl)methyl sulfamate hydrochloride (pevonedistat, Figure 1), a novel, regioselective sulfamoylation using N-(tert-butoxycarbonyl)-N-[(triethylenediaminomonomium)sulfonyl]azanide. The linear process, involving six solid isolations, has been carried out in multiple cGMP productions on 15–30 kg scale to produce pevonedistat in 98% (a/a) chemical purity and 25% overall yield.

**INTRODUCTION**

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Figure 1.

NEDD8-activating enzyme (NAE) inhibitor, has demonstrated in vitro cytotoxic activity against a variety of human malignancies and is currently being developed by Takeda Pharmaceuticals Company Limited as a clinical candidate for the treatment of cancer.1 The five-membered ring, consisting of three chiral centers and an acid/base sensitive terminal sulfamoyl group, presented considerable challenges for the development of a practical and scalable synthesis of pevonedistat.

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**Overall Process Development Strategy.** The alternate approach envisioned to support cGMP production of pevonedistat is displayed in the retrosynthesis shown in Scheme 2. Key hallmarks of this approach involved end-game installation of the chemically labile sulfamoyl group and coupling of commercially available aminodane with 4-chloropyrrolopyrimidine, derived from aminodane and commercially available aldehyde. Development of a scalable route toward the cyclopentane amine diol intermediate 4 and optimization of the chemically inefficient sulfamoylation were therefore key to enabling a scalable synthesis of pevonedistat via the proposed route and are the primary focus of this paper.

**Synthesis of Amino Diol 4.** Route scouting for alternative routes to 4 lead to two approaches which were chosen for more detailed evaluation for scale-up feasibility. The first approach utilized the Sharpless asymmetric epoxidation of 11 followed by regioselective reduction to install the desired stereo configuration of the primary and secondary alcohols (13, Scheme 3). Allyl alcohol 11, a key intermediate for asymmetric epoxidation, was prepared according to literature references with a different amine protecting group.5 The use of trityl group instead of the other protecting groups reported in the references was aimed at better stereo control over the epoxidation step. Reaction of commercially available chiral lactam 7 with SOCl₂ in MeOH afforded 8 in >95% yield after precipitation with MTBE. Trityl protection of 8 provided 9 in quantitative yield, and the resultant DCM product solution was used in the next step without isolation. Double bond migration promoted by DBU proceeded smoothly, and residual DBU was removed by extractive aqueous work-up. Solvent exchange to toluene allowed telescoping of 10 to the subsequent DIBAL-H reduction. Following aqueous work up, the desired product 11 was obtained by removal of toluene under reduced pressure, and the resulting crude oil was redissolved in DCM prior to use in the Sharpless epoxidation. The standard Sharpless epoxidation conditions, utilizing (+)-diethyl L-tartrate, produced the desired stereoisomer 12 exclusively, and the product was isolated in >80% yield after purification via column chromatography. The regioselectivity of the subsequent reductive epoxide ring opening was an obstacle for this route.

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Previous literature suggested that use of Red-Al would provide the desired product 13 in high regioselectivity. Unfortunately, no desired reaction occurred with 12 under the Red-Al conditions. A few other reducing reagents tested, such as borane–THF, borane–DMSO, LiAlH₄, and NaBH₄, resulted in mixtures of 13 and 14, favoring the formation of 14. This step was not further optimized and the mixture of 13 and 14 (1:1.3), resulting from the reaction with borane–THF as reducing reagent, was used to assess the later steps. Attempts to separate 13 from 14 by column chromatography proved difficult. In order to obtain pure 13, a protecting group strategy was employed to take advantage of the differential reactivity of the primary alcohol in the less-hindered 1,3-diol system of 13 compared to the 1,2-diol of 14. The use of 1.0 equiv of trisopropylsilyl chloride (TIPSCI) based on the amount of 13 in the mixture showed exclusive reactivity with 13 over 14 in
DCM at ambient temperature. However, separation of silylated 13 from unprotected 14 still required column chromatography. Removal of the silyl protecting group was achieved with TBAF according with 97% (a/a) purity by HPLC after column chromatography. The final removal of the trityl group was achieved via hydrogenolysis, to provide the desired chiral building block 4 in 70% isolated yield. While the route depicted in Scheme 3 proved successful in delivering 500 g of 4, low yields in the late sequence and multiple chromatographic purifications highlighted that significant process improvements would be required for larger scale production.

Following the development of the Sharpless epoxidation route, a synthesis of 4 utilizing commercially available (1R,4R)-4-tertbutoxy carbamyl amino-cyclopent-2-ene carboxylic acid (17) was explored based on a known stereospecific lactonization of cyclic or noncyclic α,β-unsaturated carboxylic acid (Scheme 4).7 Bromolactonization of 17 afforded a single stereo isomer bromolactone 18. A cosolvent system of 30% (v/v) 1,2-dimethoxyethane (DME) in water with pyridine as the base afforded sufficient solubility of 17 while allowing for precipitation of 18 from the reaction medium and easy isolation by filtration. This homogeneous aqueous/organic system also offered a cleaner purity profile (>97% a/a by HPLC) for the bromolactone 18. Major byproducts, including the pyridinium hydrobromide salt generated during the reaction, were readily removed during filtration and washing. Reductive ring opening of 18 with lithium borohydride, followed by Boc-deprotection of 19 under acidic conditions afforded 20. The Boc-deprotection step to intermediate 19 was modified by substituting hydrogen chloride with hydrogen bromide to eliminate the potential of generating mixed HCl and HBr salts. Both intermediates 19 and 20 are hygroscopic solids and were not isolated. Hydrogenation of telescoped intermediate 20 from 18 with palladium on carbon under basic conditions afforded amino-diol 4-HBr in good yield as the hydrogen bromide salt. Solvent exchange to isopropyl alcohol resulted in spontaneous crystallization of compound 4-HBr, which was isolated as a white solid by filtration in 80% yield. The success of this route mainly relies on the stereospecific...
bromolactonization of 17 and clearly demonstrated superiority over the previously described epoxidation approach to 4. The optimized manufacturing process of 4-HBr was successfully used to generate 60 kg of intermediate 4-HBr in a single batch with >95% (a/a) purity by HPLC.

Cyclization and Coupling. Formation of pyrrolo-[2,3]-pyrimidine compounds, similar to 3, by coupling of amines with acetal 21 is known in the literature. Diol 4 (or 4-HBr) underwent the same reaction smoothly with 21. In the presence of triethylamine, the amino group on intermediate 4 (or 4-HBr) reacted with 21 to effect rapid chloride displacement and spontaneous ring closure to a afford 3. Further development identified that more stable aldehyde 5, also commercially available, provided a better reaction profile under the same reaction conditions (Scheme 5). After reaction completion, direct precipitation of the product by the addition of water provided 3 in 80% yield with >98% (a/a) purity. The coupling of 6 and 3 required high energy for a fast reaction rate. Development efforts were focused on pressured reactions with various temperatures and pressure levels. Currently the reaction is performed at approximately 130 °C at 80–120 psi with 2-butanol as solvent and DIPEA as a base. Upon reaction completion, crystallization is induced by cooling and addition of MeCN/water as antisolvent, affording 2 in ca. 90% yield and >99% (a/a) purity.

Sulfamoylation. Sulfamoylation of 2 presented another significant challenge for the synthesis of pevonedistat (Scheme 2). Both primary and secondary alcohols on 2 were similarly reactive toward sulfamoylation reagents 22 and 23 (Scheme 6) used by Discovery, and a mixture of the desired product 1 with regioisomer (1') and bis-sulfamoylated byproduct (24) was produced, requiring purification of 1 by chromatography. Both approaches posed challenges to overcome with regards to efficiency10a,11 and safety11a in order to achieve successful scale-up. Sulfamoylation of mono silyl protected 2 on the secondary alcohol was also explored. However, poor selectivity during mono deprotection and necessary chromatographic purification made this approach less preferred for scale-up (Scheme 6).

Development of a regioselective sulfamoylation process began with the replacement of sulfamoyl chloride by tert-butyl carboxylsulfamoyl chloride in the hopes that the bulky tert-butyl group should provide selectivity in favor of the primary alcohol. tert-Butyl carboxylsulfamoyl chloride 25 was readily prepared by reacting tert-butanol with chlorosulfonyl...
and was directly reacted with 2 in the presence of an amine base such as triethylamine (Scheme 7). Mixing alkyl carboxylsulfamoyl chloride with tertiary amines generates inner salts, commonly known as Burgess-type reagents.13 Although widely used in synthetic chemistry,12,14 the isolation and purification of Burgess-type compounds is not trivial.14 Separation of inner salts from amino hydrochloride salts by extraction techniques rarely works as these compounds are usually unstable in common organic solvents, especially nucleophilic solvents such as water.15 A number of in situ generated tert-butyl/tertiary amine combinations were investigated for use in reaction with 2 (Scheme 8, Table 1). The tert-butyl/DABCO Burgess-type reagent was preferred for easy handling.16 Due to its reactive nature, isolation under nonaqueous conditions using a minimum number of operations was targeted. Addition of DABCO to Boc sulfonyl chloride (25) resulted in the coprecipitation of 28 and DABCO-HCl salt, where the ratio depended on the reaction solvent (Scheme 9). Attempts to separate 28 from the DABCO-HCl salt by crystallization in common solvents were unsuccessful.17 The isolation problem was solved by collecting a 1:1 solid mixture of 28 and DABCO-HCl salt (28′) from toluene in >98% yield and >98% purity by quantitative NMR analysis.16b It is worth noting that 28 has been prepared on 100–150 kg scale and stored at ambient temperature for 12 months without a detectable decrease of potency.

Using tert-Bu/DABCO Burgess-type reagent 28′, the current process for sulfamoylation of diol 2 was established and successfully carried out in multiple cGMP productions of pevonedistat (Scheme 10). Although isolable as a solid, Boc intermediate 26 was difficult to obtain in high purity and was telescoped into the sequential deprotection step. Diol 2 was consumed with 2.0 equiv of HCl, and poly-THF proved difficult to remove completely. It was later discovered that faster conversion could be obtained in higher boiling, minimally nucleophilic solvents, such as DMAC, NMP, and MeCN at elevated reaction temperatures. However, a high reaction temperature also accelerated decomposition of the Burgess-type reagents prepared in this study. MeCN was chosen as the solvent for sulfamoylation of 2 as it provided a desirable balance between reaction rate and stability of the Burgess-type reagent.

To further optimize the sulfamoylation reaction using tert-Bu/DABCO Burgess-type reagent, a robust isolation of the reagent was preferred for easy handling.16 Due to its reactive nature, isolation under nonaqueous conditions using a minimum number of operations was targeted. Addition of DABCO to Boc sulfonyl chloride (25) resulted in the coprecipitation of 28 and DABCO-HCl salt, where the ratio depended on the reaction solvent (Scheme 9). Attempts to separate 28 from the DABCO-HCl salt by crystallization in common solvents were unsuccessful.17 The isolation problem was solved by collecting a 1:1 solid mixture of 28 and DABCO-HCl salt (28′) from toluene in >98% yield and >98% purity by quantitative NMR analysis.16b It is worth noting that 28′ has been prepared on 100–150 kg scale and stored at ambient temperature for 12 months without a detectable decrease of potency.

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completely during aqueous work-up. Charcoal plug filtration followed by crystallization of the crude product in DCM afforded free base 1 in >96% (a/a) purity and >50% yield.

Salt Formation. Salt formation from 1 to pevonedistat for the Discovery route was performed after 1 was purified by chromatography. To meet GMP API purity and form specifications without chromatography, a reliable salt formation procedure that could purge baseline impurities was critical. Free base 1 was dissolved in EtOH and heated to 50 °C and seeded with 1% (w/w) pevonedistat. Further slow addition of ethanolic HCl and cooling gave the final API (pevonedistat) in high purity (>98% a/a) and the desired form.

■ CONCLUSION

A manufacturing process was developed for the synthesis of pevonedistat, a novel and potent NAE inhibitor. The chromatography free, six-step process was carried out in multiple 15−30 kg cGMP productions to a afford drug substance with greater than 98% chemical purity (Scheme 11). A high yielding chiral amino diol 4-HBr synthesis was developed through bromolactonization of 17, followed by reductive lactone opening, deprotection and removal of bromide. The four-step, two isolation process has been demonstrated on a 50 kg scale for multiple cGMP productions. This work also showcased use of a novel Burgess-type reagent in the final selective sulfamoylation step. The increase in yield and removal of chromatography for this step also contributed to the overall improvement of the synthesis of pevonedistat.

■ EXPERIMENTAL SECTION

General. All reagents and solvents were purchased commercially and used without further purification. NMR spectra were taken on either a Varian 300 MHz NMR spectrometer or a Bruker UltraShield 400 MHz NMR. All HPLC analyses were performed by using an Agilent 1100 series LC system. Melting points were obtained through DSC using TA Instruments DSC model Q2000.

HPLC Methods. A. HPLC Purity for 17, 18, and 19 (% a/a). YMC ODS-AQ, 3 μ, 4.6 × 150 mm, gradient elution with 70:30 0.1% FA in water/0.1% FA in MeCN for 22 min, with 20:80 0.1% FA in water/0.1% FA in MeCN for 3 min, 1.0 mL/min flow at 35 °C with detection at 208 nm.

B. HPLC Purity for 3 (% a/a). Waters XBridge C18, 3.5 μ, 4.6 × 150 mm, gradient elution with 85:15 0.1% NH₄OH in water/0.1% NH₄OH in MeCN for 14 min, with 30:70 0.1% NH₄OH in water/0.1% NH₄OH in MeCN for 2 min, 1.0 mL/min flow at 40 °C with detection at 270 nm.

C. HPLC Purity for 1, 2, and Pevonedistat (% a/a). Thermo Scientific Aquasil C18, 3 μ, 4.6 × 150 mm, gradient elution with 95:5 0.1% TFA in water/0.1% TFA in MeCN for 37 min, with 10:90 0.1% TFA in water/0.1% TFA in MeCN for 5 min, with 95:5 0.1% TFA in MeCN for 18 min, 0.500 mL/min flow at 40 °C with detection at 280 nm.

tert-Butyl [(1R,3R,4R,5R)-4-bromo-7-oxo-6-oxabicyclo-[3.2.0]hept-3-yl]carbamate (18). (1R,4R)-4-[(tert-Butoxycarbonyl)amino]cyclopent-2-ene-1-carboxylic acid (17, 2.00 kg, 8.80 mol) was dissolved in DME (9.60 L, 92.4 mol) and DI water (22.4 L, 1240 mol). The solution was stirred for 5
min. Pyridine (1.78 L, 22.0 mol, 2.50 equiv) was then added and the reaction stirred until the solid completely dissolved. The solution was then cooled to 0 °C, and bromine (0.567 L, 11.0 mol, 1.25 equiv) was added slowly over a period of 2 h maintaining reaction temperature at <5 °C. Once the reaction was completely determined by HPLC analysis, the reaction mixture was cooled to −10 °C. The solid formed was collected by filtration and washed with cold DI water (4.00 L, 222 mol, 0−5 °C). The solid was then suspended in DI water (20.0 L, 1110 mol, 10 vol) and stirred overnight at room temperature. The solid was filtered, washed with DI water (4.00 L, 222 mol), and dried under vacuum at 35 °C. The reaction yield was 18 (1.84 kg, 68.4%, 98.0% a/a) as an off-white solid. HPLC retention time of 18 (Method A): 16.9 min; 1H NMR (400 MHz, DMSO) δ 7.37 (d, J = 6.4 Hz, 1H), 5.20 (d, J = 4.0 Hz, 1H), 4.83 (d, J = 4.4 Hz, 1H), 4.26−4.14 (m, 1H), 4.01 (tt, J = 27.4, 13.6 Hz, 1H), 2.18−1.89 (m, 2H), 1.40 (s, 9H); 13C NMR (100 MHz, DMSO) δ 169.73, 154.99, 78.47, 77.68, 54.81, 52.27, 51.87, 28.14, 27.20; mp: 173−175 °C.

To a solution of 18 (1.31 kg) was transferred to a pressure vessel (15.2 L, 187 mol) and DI water (0.800 L, 44.4 mol) was added. The mixture was concentrated to 13.5 volumes under reduced pressure. After seed (0.2 mol %) was added, the reaction mixture was stirred for 1.5 h resulting in a suspension at 45 °C. DI water (2.00 L, 110 mol) was added to the suspension slowly over 30 min maintaining temperature at 45 °C, and then the reaction mixture was cooled to 25 °C over 2 h. The reaction was left to stir at 25 °C overnight and then cooled to 5 °C for 4 h. The solids were collected by filtration and washed with DI water (2.60 L, 144 mol). The solids were dried under vacuum at 35 °C. The reaction yield was 3 (0.636 kg, 77.5%, 99.1% a/a) as a light brown solid. HPLC retention time of 3 (Method B): 13.1 min; 1H NMR (400 MHz, DMSO) δ 8.65 (s, 1H), 7.92 (d, J = 3.7 Hz, 1H), 6.68 (d, J = 3.6 Hz, 1H), 5.48 (qd, J = 8.6, 5.4 Hz, 1H), 4.75 (s, 1H), 4.54−4.34 (m, 2H), 3.68 (dd, J = 10.2, 7.6 Hz, 1H), 3.50 (dd, J = 10.2, 6.9 Hz, 1H), 2.64−2.44 (m, 1H), 2.32−2.17 (m, 2H), 2.10 (dt, J = 13.6, 9.7 Hz, 1H), 2.02−1.85 (m, 1H); 13C NMR (100 MHz, DMSO) δ 150.49, 150.22, 149.93, 129.20, 116.93, 98.67, 71.16, 60.59, 53.45, 46.16, 42.09, 33.19; m/z: 268.4 (M + H)+; mp: 144−146 °C.

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(3) The terminal sulfamoyl group on MLN4924 was observed to be slowly displaced by chlorine from hydrochloric acid in solution, and other unidentified impurities were generated under room temperature. Any intermediate bearing the sulfamoyl group would potentially form similar impurities through nucleophilic displacement and elimination pathways. As such, it was preferable to install this functional group on intermediate 2, a late stage intermediate in the synthetic sequence.

