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ARTICLE

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Development of a Fit-for-Purpose Large-Scale Synthesis of an Oral PARP Inhibitor

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Supporting Information

ABSTRACT: Compound (**1**) a poly(ADP-ribose)polymerase (PARP) inhibitor has been made by a fit-for-purpose large-scale synthesis using either a classical resolution or chiral chromatographic separation. The development and relative merits of each route are discussed, along with operational improvements and extensive safety evaluations of potentially hazardous reactions.

INTRODUCTION

Poly(ADP-ribose)polymerase (PARP) is a ubiquitous nuclear enzyme responsible for DNA repair. PARP1 expression and activity are significantly up-regulated in certain cancers, implying an important role for this enzyme in survival and proliferation of cancer cells.[1] Inhibition of PARP activity has demonstrated antitumor effects in several cancers, particularly those with deective BRCA-1 and -2 repair molecules.[2] Compound (**1**) (Figure 1) was recently identified by Merck Research Laboratories as a potential orally active PARP-1 inhibitor which demonstrated efficacy as a single agent in a xenograph model of BRCA-1-deficient cancer.[3] On the basis of these encouraging results the development of a synthesis of compound **1** suitable for large-scale implementation to support additional preclinical and early clinical studies was required.

Compound (**1**) contains an unusual 2*H*-indazole moiety[4] attached to a 3-aryl piperidine which bears the compound's single chiral centre, and installation of these functional elements was expected to present a significant synthetic challenge. The Medicinal Chemistry approach to compound **1** is shown in Scheme 1. The racemic piperidine **2** was accessed by reduction of the 3-aryl pyridine **3** and then resolved by salt formation with tartaric acid. Protection of the piperidine nitrogen in enantiomerically upgraded piperidine **2** and condensation with aldehyde **4** afforded imine **5** which, after displacement of the nitro group with sodium azide, underwent a thermally promoted cyclisation to afford the 2-aryl indazole **6**.[5] Conversion of the ester functionality to a primary amide and deprotection afforded the active pharmaceutical ingredient (API) as the hydrochloride salt. A final chiral HPLC purification was then required to upgrade the enantiomeric purity to >98% ee, followed by lyophilization to give the desired compound **1** as an amorphous HCl salt.

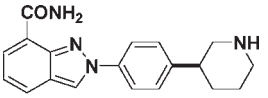

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Figure 1. Structure of compound **1**.

The team recognised this route as a relatively short synthesis to such a complex molecule, and a viable approach on the basis of the time constraints at this stage of development. However, prior to larger-scale implementation a number of issues would need to be addressed, particularly surrounding safety and throughput considerations. First, although the synthesis of the racemic piperidine **2** appeared straightforward, the resolution was both low yielding and inefficient as, even after multiple recrystallisations of the diastereomeric salt, a chiral HPLC purification was still required later in the synthesis. Second, a number of safety concerns existed around the formation and reaction of the intermediate azide **7**, especially given the elevated temperature employed for this transformation. A third significant issue was the lack of a suitable final crystalline form; the previously employed HCl salt was shown to be highly hygroscopic, requiring isolation by lyophilization as an amorphous foam. The synthesis of aldehyde **4** (*vide infra*) also contained low-yielding and environmentally inappropriate steps. Finally most intermediates had been purified by column chromatography, undesirable on large scale.

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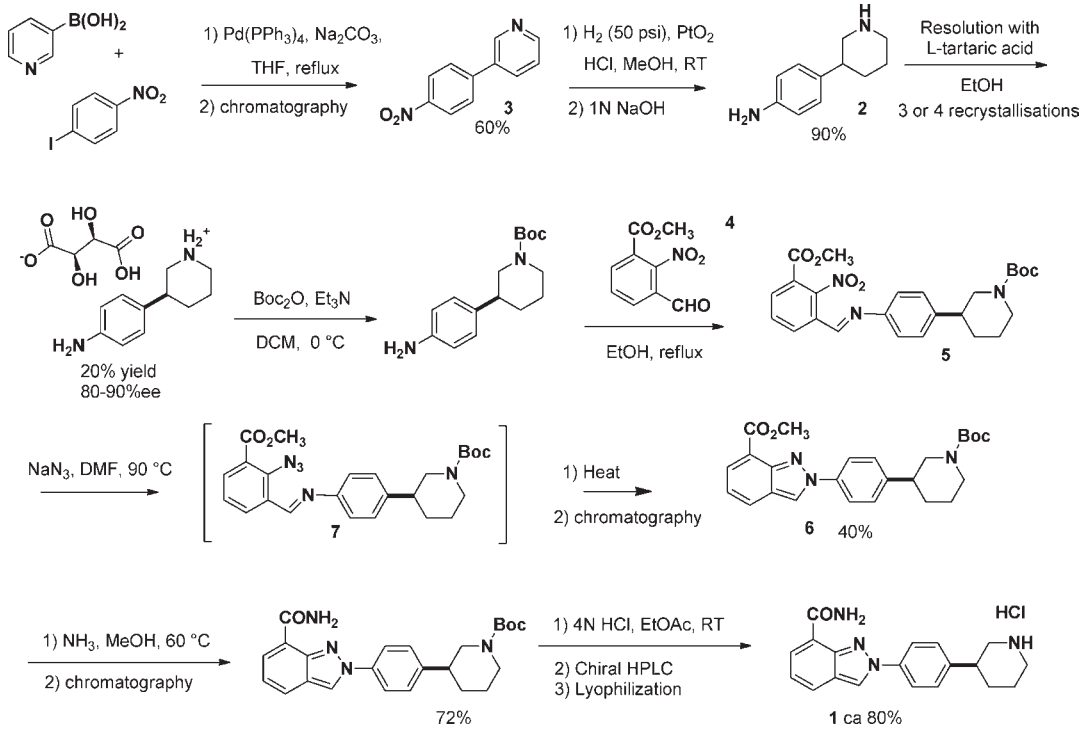
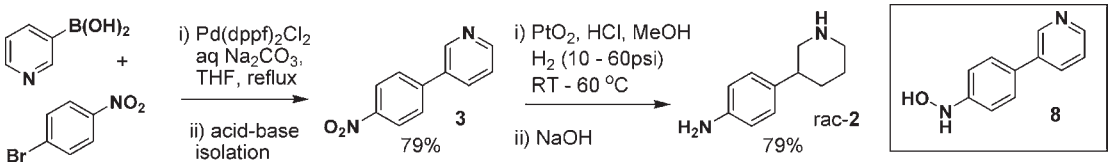
Scheme 1. Medicinal Chemistry approach to compound 1



Scheme 2. Synthesis of racemic piperidine 2



RESULTS AND DISCUSSION: RESOLUTION APPROACH

Synthesis of Piperidine 2. The Medicinal Chemistry synthesis of compound **1** started with a Suzzuki coupling between 1-iodo-4-nitrobenzene and pyridine 3-boronic acid to give **3**.[6] The reaction required an excess of the boronic acid and 5 mol % of the notoriously air sensitive palladium tetrakistriphenylphosphine as catalyst. Optimisation of solvent, base and catalyst conditions identified a method which allowed for use of the cheaper 1-bromo-4-nitrobenzene as starting material, along with a reduced loading of $PdCl_2$(dppf) (2 mol %) and only a slight excess (1.05 equiv) of boronic acid (Scheme 2). Incorporation of a carbon treatment and extraction of the desired product into aqueous acid efficiently removed phosphine and other impurities prior to isolation from the aqueous layer by neutralisation in 79% yield. In this way over 17 kg of the nitropyridine product **3** was prepared in a single batch.

Reduction of both the nitro group and pyridine in compound **3** had previously been carried out at high dilution in acidified methanol using 9 mol % of PtO_2 and 50 psi hydrogen. This procedure raised safety concerns on the bases of the initial exothermic reaction and also the potential for buildup of hydroxylamine intermediates such as **8**.[7,8] It was found that the initial reaction exotherm could be controlled by starting the reaction at a lower temperature and pressure until initial hydrogen uptake had slowed (nitro reduction complete), followed by an increase in both temperature and pressure to complete the pyridine reduction. Differential scanning calorimetric (DSC) evaluation of a partially reduced reaction mixture containing a high quantity of hydroxylamine intermediate **8** showed no exothermic activity up to 250 °C removing most concerns about this intermediate. Further optimisation allowed for reaction volume in methanol to be reduced to 10 mL/g, with concentrated hydrochloric acid favored as the acid source. A range of other catalysts was explored, including iron- and vanadium-doped platinum catalysts (which could avoid buildup of hydroxylamine intermediate **8**), but none were found to be superior to PtO_2, and to retain reasonable reaction rates the loading remained at a relatively high 7 mol %. After complete reaction, the mixture was diluted with water to ensure the product remained in solution during catalyst filtration, the methanol was removed under vacuum, and the aqueous slurry was neutralised with NaOH and extracted into isopropyl acetate (IPAc), prior to isolation from an IPAc/heptane mixture to give the product rac-**2** in 79% yield on 17-kg scale (Scheme 2).

Resolution of Piperidine 2. A range of chiral acids were screened as potential resolving agents for racemic **2**, however a limited number of solid salts were obtained.[9] The most promising

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results were from tartaric acid derivatives and thus dibenzoyl tartaric acid (DBT) was chosen for further development. The piperidine **2** was shown to form a racemic hemi (2:1) salt on reaction with 0.5 equiv of acid, a partially resolved 1:1 salt on reaction with 1 equiv of acid, and a partially resolved bis (1:2) salt *with the opposite sense of induction* on reaction with 2 equiv of acid. As such for an effective resolution a clear understanding of the relative stabilities/solubilities of these salts and careful control of stoichiometry would be required.

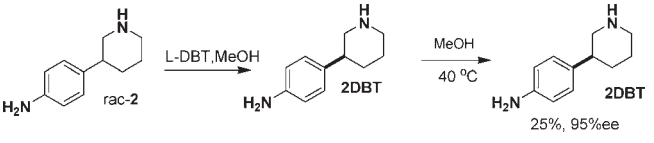
Solubility studies on the 1:1 salt indicated that there were few physical differences between the desired and undesired diastereomeric salts in a range of solvents, and that any resolution was largely kinetically controlled. Indeed, the initially obtained enantiomeric excess was shown to decrease after an overnight age, leading to racemic material. Under optimal conditions of slow addition of the amine to a solution of D-dibenzoyl tartaric acid (D-DBT) in methanol followed by addition of ethyl acetate as antisolvent, yields of ~38%, of 65−70% ee could be obtained. Although higher recoveries were obtained with less methanol or more ethyl acetate, the enantiomeric excess was lower. The 1:1 DBT salt could be up-graded from 67 to 69% ee to over 90% ee by heating in methanol (10 mL/g) however recoveries were below 50% and it became clear that obtaining a satisfactory yield using this salt would not be possible. Instead we began to examine use of the bis-DBT salt formed from L-DBT.

Samples of the two diastereomeric bis-DBT salts were prepared from enantiomerically pure amine (generated by semi-preparative chiral chromatography) to allow for characterization of physical properties. Both salts were confirmed as crystalline compounds on the basis of XRPD. Melting points were found to be essentially identical (182.7 °C for desired and 182.2 °C for the undesired) with the desired salt giving an endotherm at 60 − 80 °C indicating a possible solvate. The solubilities were found to be very similar in a range of solvents, although in alcoholic solvents the desired salt was marginally less soluble than the undesired, suggesting that a resolution might be possible, although on the basis of the modest differences some reliance on kinetics would still be required (Table 1). Given the better solubility in methanol, especially at higher temperatures this solvent was chosen for further development.

Table 1. Solubility of 1:2 DBT salts (solubility expressed as mg/mL for amine free base)

solvent	desired L-DBT salt (mg/mL)	undesired salt (mg/mL)
MeOH	5.5	8.2
MeOH, 35 °C	7.8	12.4
MeOH, 45 °C	10.1	18.2
EtOH	1.1	1.7
THF	4.2	5.3
MeCN	<1	<1
Acetone	4.2	2.5

Scheme 3. Resolution of piperidine 2

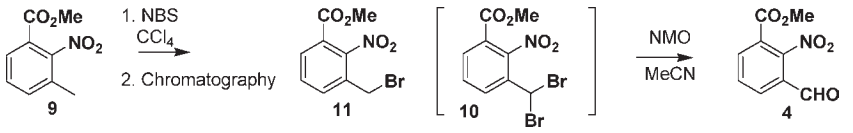


Using around 30 volumes of MeOH and 2.2 equiv of L-DBT, a 35% yield of 84% ee material could be obtained; however, as for the 1:1 salts the enantiomeric excess decreased over time. It was found that heat/cool cycles of the initially formed salt could boost the ee and filtration at 30−40 °C allowed for isolation of higher ee material. Further cooling and/or aging of the salt slurry prior to filtration led to lower selectivity, although NMR analysis confirmed the compound was still the desired bis-salt. Notably, XRPD analysis of a range of samples of varying ee did not match the pure diastereomeric salt previously prepared, nor was a consistent pattern seen between such samples, suggesting that even small amounts of the undesired enantiomer led to mixtures of crystalline forms. A number of methods to upgrade the ee of the salt by reslurry or recrystallizations were investigated; however, only the use of MeOH as solvent led any chiral upgrade. Once again higher temperatures were found to be more productive, and after a reslury at 40 °C 86% ee salt could be upgraded to 98% ee, in 77% recovery on laboratory scale. The resolution was run using 6.0 kg of racemic amine. Unfortunately, on this scale, control of time cycles in the initial resolution led to slightly lower yields and ee, but after the upgrade an overall yield of 25% of the bis-dibenzoyl tartaric acid salt of (*R*)-**2** (1.5 kg of amine free base) in 95% ee was obtained (Scheme 3).

Synthesis of Aldehyde 4. Aldehyde **4** was initially prepared by bromination of ester **9** with *N*-bromosucinimide in carbon tetrachloride which led to a mixture of products, including the major impurity bis-bromo **10**. This required purification by chromatography to afford clean **11** (Scheme 4). The benzylic bromide **11** was then oxidized with 4-methylmorpholine *N*-oxide (NMO) in the presence of molecular sieves to give aldehyde **4** which was used as a crude solid after evaporation to dryness in the subsequent imine formation. The issue of solvent, reaction byproduct, and the need for chromatography would need to be addressed for larger-scale implementation.

Other solvents could be used (MeCN or IPAc) for the bromination in place of the carbon tetrachloride, and the product could be crystallized from ethanol, however, bis-bromination still led to significant quantities of dibromo compound **10** and a modest 45% yield. Treatment of the mixture of bromides **10** and **11** with diethylphosphite and triethylamine prior to isolation was effective in converting dibromo **10** to bromide **11**, leading to an increased yield of 60% at the expense of an extra processing step, however careful control of reagent charge was required as this protocol also led to some further debromination to starting material **9**. On the basis of these issues it appeared the bromination procedure would likely not be robust on scale, and an alternative was sought.

Scheme 4. Initial synthesis of aldehyde 4



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Reaction of ester **9** with dimethylformamide dimethyl acetal (DMF−DMA)[10] in DMF afforded enamine **12** which was precipitated by the addition of water. This procedure was operationally straightforward, obviated the need for any extractive workups, and proceeded well on large scale to give a 70% isolated yield of the enamine. Conversion of enamine **12** to aldehyde **4** was achieved by reaction with sodium periodate in DMF/water to give, after a carbon treatment and crystallization from ethyl acetate, an 80% yield of the desired aldehdye in high purity (Scheme 5). On the basis of the improved yields and ease of operation of this sequence this route was chosen for long-term synthesis of the aldehyde fragment and has subsequently been run on 100-kg scale.[11]

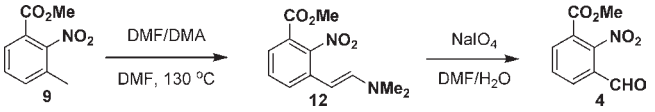
Indazole Formation. The next synthetic operation required was the conversion of piperidine **2** to indazole **6** via the intermediacy of imine **5**. To ensure clean imine formation, Boc-protection of the piperidine nitrogen in **2** was found to be necessary, and use of ethanol as solvent allowed for a one-pot protection/imine formation to be carried out. Hence, salt break of the bis-dibenzoyl tartaric acid salt and extraction into CH_2Cl_2 followed by solvent switch to ethanol afforded a solution of (*R*)-**2** suitable for use in this reaction. Addition of Boc_2O at 0 °C followed by the aldehyde **4** at room temperature and heating to reflux for 6 h led to formation of the desired imine **5**, which could be isolated in in 69% yield on 2.0-kg scale after cooling and filtration (Scheme 6). A significant impurity in the process was found to be the double Boc-protected piperidine **13**, which was difficult to reject at this stage, leading to its being incorporated in the isolated solid along with imine **5**, thus lowering the yield.

As previously highlighted, in the original synthesis conversion of imine **5** to indazole **6** was carried out using 1.05 equiv of sodium azide in DMF at elevated temperature (90 °C) for three days leading to a mixture of the ester **6** along with 5−15% of the corresponding carboxylic acid **14** (Scheme 7).[5] Column chromatography of the concentrated reaction mixture afforded the product **6** in around 50−55% yield. These conditions were found to perform as expected and could be accelerated by increasing the reaction temperature to 110 °C which also allowed for a reduction in azide charge to 1.0 equiv. With the use of mass spectral analysis the reaction appears to proceed *via* the azide intermediates **7** and **15**. The amount of acid **14** present in the final reaction mixture mirrors the acid:ester ratio seen at the azide intermediate stage. This implied that ester hydrolysis occurred before the azide displacement step, and it is thought that nitro-group-assisted ester hydrolysis of the starting imine can occur under the reaction conditions.[12] While we were pleased to verify the cyclization conditions did indeed afford the desired compound, it was clear that some significant improvements and hazard evaluations would be required for safe and efficient large-scale implementation.

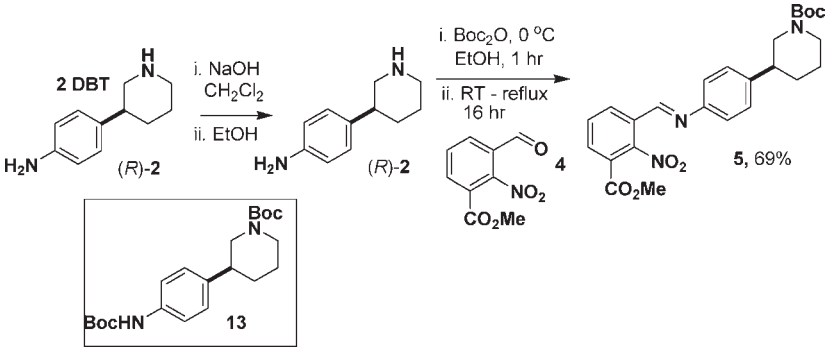
Initial hazard testing focused on evaluation of hydrazoic acid levels in the head space of the reaction or workup mixtures, as this compound is both highly toxic and can form explosive gas mixtures with oxygen or nitrogen above concentrations of 8−12%.[13] Some variation in the lower decomposition level of hydrazoic acid has been reported; however, a recent study appeared to confirm that detonation below 10 vol % should not be possible.[13b] Maintaining a basic pH during the reaction mixture should ensure that any hydrazoic acid remained ionized and would thus limit levels in the head space and as such was considered desirable. After some screening it was found that addition of 1 equiv of 2,6-lutidine to the reaction mixture served to maintain a basic pH and had no detrimental effect on the reaction profile or rate. Hence, this base was added prior to heating any reactions. Headspace monitoring using FT-IR indicated undetectable levels of hydrazoic acid in the headspace throughout the course of the reaction using a nitrogen sweep of 16 mL/min. On the basis of comparison with CO_2 in the headspace and the detection limit of the instrument, this data implied a worse-case hydrazoic acid level in the headspace of 60 ppm with this nitrogen sweep, and it was clear that only a very modest nitrogen sweep (about 0.02 mL/min) would be required to keep concentrations of hydrazoic acid below 5 vol % which compares favorably with the lower decomposition limit of 10 vol %. As such there was no significant risk with this aspect of the reaction. During workup development (*vide infra*) it became clear than an acidic wash would be required, and hence, this scenario was also evaluated. After complete reaction the DMF mixture was cooled and partitioned between THF and water, followed by a brine wash of the organic layer. The THF layer was then treated with 2.0 M aq HCl, and once again headspace was monitored by FT-IR for generation of hydrazoic acid. Despite the acidic conditions, once again levels of hydrazoic acid were undetectable, indicating that all azide had been consumed or removed to the aqueous washes prior to the acid treatment.

A second battery of hazard evaluation was carried out to assess the thermal hazards of the reaction. On the basis of DSC analysis, the imine starting material **5** (as a solid) was found to have a high

Scheme 5. Large-scale synthesis of aldehyde 4



Scheme 6. Synthesis of imine 5



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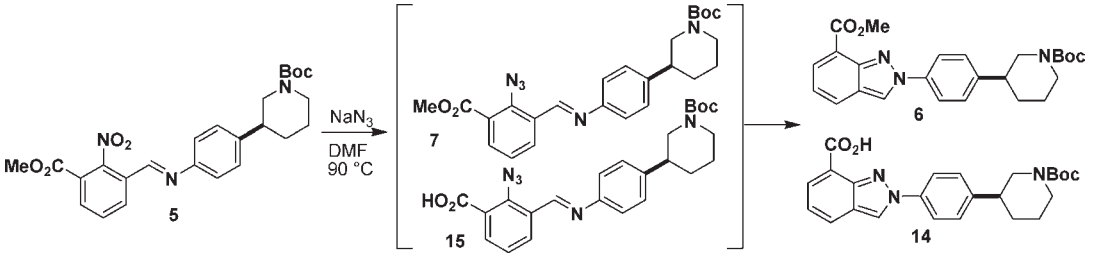
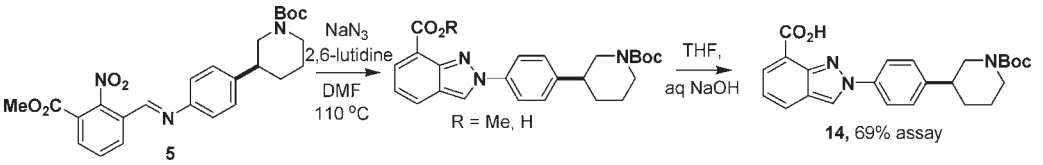
Scheme 7. Initial synthesis of indazole 6



Scheme 8. Large-scale indazole formation



thermal energy of 206 cal/g initiating at 148 °C but was not shock sensitive. The prereaction mixture of imine, sodium azide, and 2,6-lutidine in DMF also showed an exotherm (9.5 cal/g) initiating at 125 °C which was very close to the proposed operating temperature of 110 °C; hence, additional testing using accelerating rate calorimetry (ARC) was performed. These experiments indicated an adiabatic temperature rise of 11−12 °C during formation of the indazole, and although self-heating could occur at just above the reaction temperature, the high boiling point of DMF meant a runaway exotherm would not be likely and temperature could be controlled by jacket cooling. The maximum pressure rise was also calculated (0.4 psi/min) which could also be easily managed by vessel venting without the need for additional controls. Any exothermic activity during the workup procedure was found to initiate well above the proposed operating temperature for these subsequent operations. On the basis of the positive results from the above safety tests it was felt that a path forward could be found using the sodium azide-mediated indazole reaction, and thus attention reverted to process optimization, isolation, and purification.

On small scale the indazole ester had been isolated by direct chromatography after concentration of the DMF. Clearly this was not desirable on scale, and hence an aqueous workup and more appropriate isolation were evaluated. The use of THF along with half-saturated brine led to good aqueous/organic cuts, and further brine washes allowed for removal of most of the DMF, leaving a solution of ester **6** and acid **14** in THF. Given that the next step in the synthetic sequence was the introduction of a primary amide in place of the ester, it was desirable to convert this mixture to a single species to avoid yield loss. Moreover, as formation of the amide from the ester **6** involved heating with ammonia/methanol in a sealed tube, it was felt that the related acid **14** might be a better intermediate allowing for amide formation under more standard and mild conditions *via* acid activation. Hence, conversion of the acid/ester mixture to the pure acid **14** by basic hydrolysis was explored. Exposure of the THF solution of compounds **6** and **14** to 2.0 M NaOH and heating to 35 °C were sufficient to convert remaining **6** to **14**. After layer separation and wash with acidified brine, a dark solution of the acid **14** was obtained in ~70% assay yield from the imine **5**. With these modifications in place the indazole formation/ester hydrolysis was successfully run on 2-kg scale without incident to give a 69% assay yield of the acid (Scheme 8). Attempts to isolate the acid **14** by crystallization did not prove fruitful, and thus the solution was used without further purification in the amide formation.

Completion of Synthesis. Surprisingly, the attempted formation of amide **16** from acid **14** under a variety of standard activation conditions (acid chloride, CDI, EDC, etc.) followed by reaction with ammonium hydroxide did not prove to be successful, due to either cleavage of the Boc protecting group (under acid chloride conditions) or low reactivity of the hindered acid. However, activation by Boc_2O in the presence of pyridine and then reaction with ammonium bicarbonate gave clean conversion to the amide in 24 h, and these conditions (subject to use of excess reagents) were found to be suitable for use with the crude acid stream generated as described previously (Scheme 9). After aqueous workup and solvent switch the amide could be isolated from IPAc, containing a number of impurities (and color which had not been removed from the previous step) of which the most significant was ~5% of bis-Boc piperidine **13**. A reslurry with methyl *tert*-butyl ether (MTBE) removed these impurities; however, to give high-purity amide, fairly significant yield losses were incurred that led to isolation of the amide **16** in only 52% yield (based on assay of acid **14**) on 1.5-kg scale, and alternate purification methods would need to be considered for subsequent deliveries.

The API had previously been isolated by lyophilization as an amorphous and hygroscopic HCl salt for early studies. For practical purposes, as the compound entered development, a more stable and preferably crystalline salt form was desirable. High-throughput acid salt screening of the free base using SYMMX systems identified potential crystalline salts resulting from fumaric, sulfuric, benzenesulfonic, and *p*-toluenesulfonic acids.[14] On the basis of these results, use of a single acid to achieve both the final Boc-deprotection and generate a pharmaceutically acceptable salt in one operation appeared feasible. Indeed, after further optimization the use of *p*-toluene sulfonic acid in THF/water at 60 °C gave clean removal of the protecting group with concomitant precipitation of the related salt as a crystalline monohydrate in high yield. In contrast to the previously used hydrochloride salt, this compound

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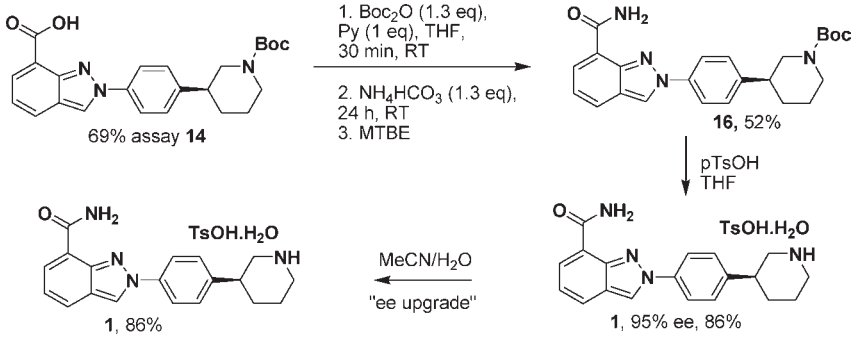
Scheme 9. Completion of the synthesis



was stable and nonhygroscopic and had suitable properties for long-term development. In this way on kilogram scale the *p*-toluenesulfonic acid salt of **1** was generated in high chemical purity and good yield in a single step from amide **16** in 86% yield (Scheme 9).

Unfortunately, the enantiomeric purity of **1** at this stage was found to mirror that of the aniline **2** (95%ee) obtained from the resolution, indicating that no significant upgrade had occurred at any of the interim isolated intermediates; hence, further processing was required to afford acceptable material. The medicinal chemists had employed a chiral HPLC separation of API to achieve this goal; however, given the low solubility of the compound, employment of such a procedure for kilogram-scale deliveries was unattractive, and so a reslurry or crystallization procedure was sought. A number of solvents were evaluated to this end; however, the low solubility of the salt in most systems limited options. After some experimentation it was noted that the solubility of the salt in a 1:1 acetonitrile/water mixture (22 mg/mL) was noticeably higher than solubilities in acetonitrile (0.67 mg/mL) or water (0.77 mg/mL). Additionally after a reslurry in this 1:1 mixture the filtrate showed an increased enantiomeric excess, while the isolated solid was downgraded. On the basis of this observation an upgrade, involving removal of racemic or low ee material followed by isolation from the filtrate, appeared viable. Optimization of solvent volume allowed for isolation of the initial solids in 4−6% yield and with about 30% ee, with the filtrate showing >99.5% ee. Distillation of the acetonitrile from the filtrate afforded a white slurry, and after isolation this salt showed excellent enantiopurities, typically >99.5% ee with good isolated yields (85% on 100 g scale) and was confirmed to be the desired tosylate monohydrate salt.

In this way a delivery of ∼500 g of high chemical (>99%) and enantio (>99.5%) purity API was prepared in a timely manner to provide material for toxicology studies. We achieved our initial goals for the synthesis by (i) replacing the chiral chromatography with a resolution/crystallization, (ii) evaluating safety aspects of the indazole formation and defining safe operating conditions, (iii) changing the aldehyde synthesis to improve yields and reduce environmental impact, (iv) elimination of the use of chromatography by substituting acid/base extractions or suitable crystallizations, and (v) defining a stable salt for long-term development.

CHIRAL SEPARATION APPROACH

The procedures outlined above allowed for the rapid generation of hundreds of grams of API to initiate the development program for compound **1**; however, it would not be a viable approach for subsequent deliveries of multikilogram amounts. The overall yield for the process was a modest <3% with a number of issues identified. The classical resolution was low yielding (25% overall yield), appeared to operate under kinetic control, and only afforded material of 95% ee, necessitating a subsequent upgrade at the API stage. Second, a lack of control in the Boc-protection of the piperidine **2** led to a modest yield (69%) for an apparently straightforward reaction, while the bis-Boc impurity **13** carried through a number of steps and impacted later yields. Finally, the indazole cyclization generated a highly colored solution, and the need to remedy this in the subsequent amidation step significantly impacted that isolation (75% assay, but 52% isolated for the amidation of acid **14** to amide **16**). Since a rapid follow-up delivery of multikilogram quantities was needed, complete re-engineering of the synthesis was not a viable option, and instead we focused on addressing the shortcomings noted above.

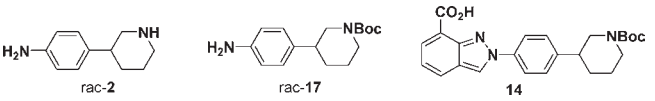


Figure 2. Potential intermediates for resolution.

Initially the classical resolution of piperidine rac-**2** was re-evaluated, with a view to converting the >10 kg of racemic material that had been set aside from the first delivery. However, despite extensive screening it did not prove possible to improve on the initial results using DBT. Attempts to resolve other intermediates (such as Boc-protected piperidine **17** or acid **14**) were also unsuccessful (Figure 2).

With this in mind the viability of a large-scale chiral separation was reconsidered as an interim solution until an asymmetric approach to the molecule was available. On the basis of relative solubilities and preliminary screening, the Boc-protected piperidine, rac-**17**, was identified as a suitable candidate for separation on a Chiralpak AD column using ethanol/heptane. Modeling the use of single injections on a 0.46 cm × 250 cm AD 20 μm column allowed for predictions of solvent volumes and separation time required for a variety of different column diameters. Although the compound had good solubility in ethanol, tight separation coupled with the the need for the desired enantiomer being eluted second meant an injection cycle time of 10 min was required, and this contributed to the modest productivity estimate of 0.27 kkd (Kg product separated per Kg stationary phase per day). To achieve reasonable cycle times a 30-cm column was selected as suitable for separation of multikilogram quantities.

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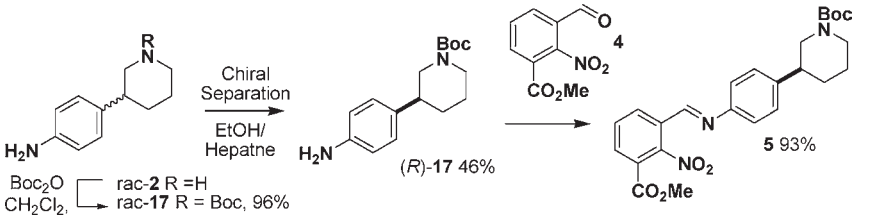
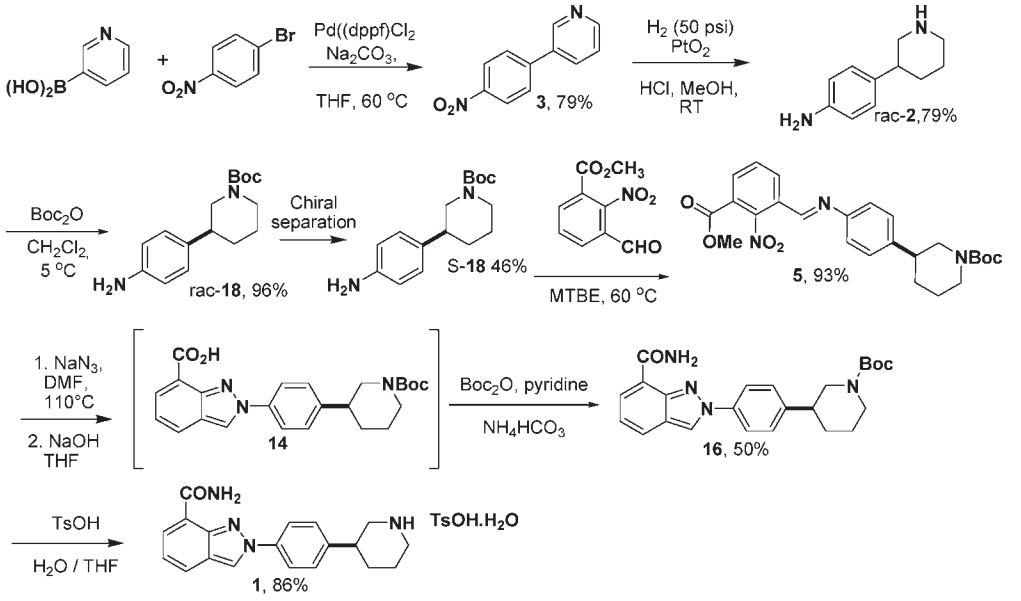
Scheme 10. Improved imine formation via separation of 17



Scheme 11. Optimized synthesis of 1



With the separation of protected piperidine **17** confirmed as the path forward some minor changes around the Boc-protection and imine formation chemistry were required to support isolation and use of this intermediate. Evaluation of reaction solvents for conversion of rac-**2** to rac-**17** indicated that formation of bis-Boc impurity **13** was accelerated in alcoholic solvents (such as ethanol which had been previously employed) but could be minimized by use of dichloromethane as solvent, with the product being isolated in high purity and 96% yield via crystallization from isopropanol/water. Chiral separation of 8.5 kg of rac-**17** proceeded as planned to give a 92% recovery (46% yield) of the desired enantiomer in 99.3% ee. With isolated (*R*)-**17** in hand, MTBE was found to be a better solvent than ethanol for the imine formation in regard to impurity formation and improved filtration properties with an isolated yield of 93%. In this way 8.5 kg of rac-**2** was converted to 6.0 kg of imine **5** in 40% yield, a significant improvement from the 17% yield obtained in the prior process (Scheme 10).

Further improvements to the sequence were realized during optimization of the indazole formation/amidation sequence. While the chemical reactions scaled well, isolation of the amide proceeded with significant yield loss as a result of the need to reduce color and other impurities. Treatment of the crude stream with silica gel during the amidation workup led to greatly improved isolation properties and an increased yield of 52% on 5-kg scale for the three-step sequence compared to the former 37%. Final Boc-deprotection and salt formation proceeded as expected to give the desired salt directly in high chemical and enantiopurity. On the basis of the greater efficiency of the chiral separation vs the resolution and the improvements to a number of reactions and isolations, the synthesis of **1** from commercial starting material now proceeded in an 11% yield (Scheme 11) and allowed for generation of multikilogram quantities of material to support the program into initial clinical studies.

CONCLUSION

We have described two approaches to the PARP inhibitor **1**, one relying on classical resolution and one on a chiral separation. The yield obtained for the separation route was significantly better than for the chiral resolution on the basis of the higher recovery and chiral purity obtained in the separation. This approach was found to be optimum for API deliveries of up to 5 kg. For yet larger deliveries the modest throughput in the chiral separation would become limiting, and the need to define an asymmetric approach to the piperidine is apparent. Work towards this goal will be reported in a subsequent communication.

EXPERIMENTAL SECTION

General. HPLC monitoring of most reactions was carried out with the use of commercially available reverse-phase columns (Zorbax Eclipse XDB-C8 or Phenomenex LUNA C18) eluted

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with 0.1% H_3PO_4 (aq) and acetonitrile. The chiral purity of amine **2** was determined using a Chiralpak OJ-H, 5.0 μm, 250 mm × 3.0 mm column, eluting with 60:40 hexane/EtOH + 0.1% $iBuNH_2$. Imine formation was followed using a Waters Xbridge C18, column eluting with 10 mM ammonium carbonate in water adjusted to pH 9 with ammonium hydroxide and acetonitrile. Chiral purity of the API was determined using Chiralpak AS-H, eluting with 60:40 hexane/EtOH + 0.1% $iBuNH_2$. HPLC assay yields were obtained using pure compounds as standards. Isolated yields refer to yields corrected for purity on the basis of HPLC assay using purified standards. Reactions were run under a nitrogen atmosphere. All reagents and solvents were used as received without further purification.

Subsequent to completion of this work an occupational exposure limit of 0.1 $\mu g/m^3$ for the API was recommended on the basis of potency, in vivo genotoxicity results, and mode of action. An occupational exposure limit of 1.0 $\mu g/m^3$ was recommended for the penultimate amide **16**.

3-(4-Nitrophenyl)pyridine (3). A solution of 3-pyridineboronic acid (14.7 kg), 1-bromo-4-nitrobenzene (23.0 kg), and THF (325 kg) was treated with 2.0 M sodium carbonate solution (166 L) and $Pd(dppf)Cl_2$ (1.67 kg). The mixture was thoroughly degassed and heated to reflux (66 °C) for 2 h after which time HPLC analysis indicated complete (>99%) reaction. The batch was cooled to 30 °C, ethyl acetate (88 kg) was added, and the mixture was filtered through a polypropylene filter cloth, washing with additional ethyl acetate (88 kg). The filtrate was returned to the vessel, the lower aqueous layer was removed, and the organic layer was passed through a CUNO filter with a R53SP16 cartridge. The ethyl acetate layer was extracted with 2.0 M hydrochloric acid (250 L), and the aqueous was washed three times with ethyl acetate (3 × 80 kg) to aid complete removal of all nonbasic impurities. The acidic aqueous layer was then neutralized with 10 M sodium hydroxide solution (78 L), keeping the temperature at <30 °C until a final pH of around 7−8 was attained and a white slurry generated. The solids were filtered and washed with water (50 L) and dried in vacuo at 55 °C to afford the desired 3-(4-nitrophenyl)pyridine **3**, 17.19 kg (79% yield).

Spectroscopic data were in accord with previously published data.[3a,6]

4-(Piperidin-3-yl)aniline (rac-2). 3-(4-Nitrophenyl)pyridine (**3**; 8.60 kg) was charged to a 160-L hydrogenation vessel and inerted. Methanol (57.4 L), 5.0 M HCl, and then a slurry of PtO_2 (600 g) in methanol (10 L) were added, using minimal vacuum. The vessel was then reinerted with nitrogen, and the stirred reaction mixture was cooled to ∼10 °C. The stirrer was stopped, and the vessel was evacuated and then filled with hydrogen to a pressure of 1 bar. The stirrer was then started, the reaction mixture was left to age under these conditions until the exotherm subsided, and the rate of hydrogen uptake slowed. At this stage the hydrogen pressure was increased to 4 bar, the reaction mixture was warmed to 40 °C and stirred for 16 h, after which time HPLC analysis indicated >95% conversion, and hydrogen uptake had ceased. The vessel was inerted with nitrogen, water (43 L) was added to the stirred batch, and the batch was cooled to 20 °C. The batch was filtered through a 1 μm cartridge filter which was washed with 2:1 methanol/water (15 L).

Two batches at the above scale were run before being combined for further processing.

The aqueous methanol solution was concentrated *in vacuo*, keeping the temperature below 35 °C to a volume of ∼120 L. Isopropyl acetate (160 L) and 5.0 M sodium hydroxide solution were added, the resulting biphasic mixture was stirred for 15 min, and the two layers were then separated. The aqueous layer was re-extracted with isopropyl acetate (80 L), and the two organic layers were combined and concentrated under partial vacuum to ∼55 L, whilst maintaining the internal temperature at 30−40 °C. Heptane (27 L) was then added, and the batch was reconcentrated to 55 L. A second portion of heptane (28 L) was added, and the batch was again concentrated to 55 L. A final portion of heptane (10 L) was added, and the stirred slurry was then cooled to 17 °C, filtered, and washed with 3:1 heptane/isopropyl acetate (16 L). The resulting wet cake was dried to afford piperidinylaniline rac-**2** (11.8 kg, 79%).

Spectroscopic data in accord with previously published.[3a,3c,15]

***tert*-Butyl 3-(4-aminophenyl)piperidine-1-carboxylate (rac-17).** Piperidine rac-**2** (5.69 kg) and dichloromethane (60.5 kg) were charged to a vessel and cooled to 0−5 °C. Di-*tert*-butyl carbonate (6.98 kg) was dissolved in dichloromethane (30.3 kg) and added, keeping the batch temperature below 10 °C. The batch was aged for 30 min at 0−5 °C, after which time HPLC analysis indicated <0.5% starting material remained. Water (22.8 kg) was charged, the mixture was warmed to room temperature, and the layers were then separated. The organic layer was distilled to a volume of ∼20 L, isopropanol (IPA) (53.6 kg) was added, and distillation continued to a volume of ∼35 L, containing <1% of DCM relative to IPA. The solution was cooled to 45 °C and seeded with front run material. After one hour water (63 kg) was added over 2 h keeping $T \approx 40-45$ °C. The slurry was cooled slowly to 15 °C, aged for a further hour, and filtered, washing with a mixture of water/IPA (11 kg water, 4 kg IPA, premixed), and the solid was dried in a 55 °C vacuum oven to give the product rac-**17** as an off-white solid (8.65 kg, 96%).

Spectroscopic data were in accord with previously published data.[3a,c]

Separation of rac-18. A 90 mg/mL solution of rac-**17** was prepared by dissolving 8.50 kg of rac-**17** in ethanol (95 L). This was separated on a 30 cm internal diameter Chiralpak AD (20 um) column using 480 mL stacked injections at 10 min intervals and eluting with 5% ethanol/heptane. After separation the desired fractions were combined, concentrated to a low volume, and solvent switched to MTBE and concentrated to a final volume of 3.0 L. This was heated to 48 °C for one hour, and then heptane (4 L) was added and heating continued for one hour. The slurry was cooled to 10 °C over an hour and then was filtered, washed with 1 L of 20% MTBE in heptanes, and dried at 50 °C in a vacuum oven to give (*R*)-**17** as a beige solid (100 LCAP, 100 LCWP, 3.91 kg, 46%).

Methyl 3-(2-(Dimethylamino)vinyl)-2-nitrobenzoate 12. To a solution of methyl ester **9** (130.0 kg) in DMF (585 kg) was added DMF−DMA (286 kg) and the resulting solution warmed to 130 °C and stirred for 30 h or until <3% starting material remained. The solution was cooled to 10 °C and water (1300 kg) added dropwise over one hour, keeping the internal temperature below 20 °C. The resulting slurry was aged for one hour and then filtered, and the solids were washed with water (200 kg) to afford 120.4 kg of enamine **12** (66.9% yield).

Spectroscopic data were in accord with previously published data.[16]

Methyl 3-Formyl-2-nitrobenzoate 4. A solution of enamine **12** (111.5 kg) in DMF (718 kg) at room temperature was added dropwise to a solution of sodium periodate (237.2 kg) in water (930 kg) at 45 °C over a period of one hour, and then the resulting mixture was stirred at the same temperature for 2 h, or

until HPLC analysis indicated complete reaction, and then was cooled to 5 °C. Water (1560 kg) was added over one hour, and the slurry was stirred for 2 h at 5 °C before filtering and washing with further water (500 kg). The wet cake was dissolved in ethyl acetate (1077 kg). DARCO G60 (44.6 kg) was added and the mixture heated to 75 °C and stirred for 2 h. The batch was cooled to 55 °C, filtered, and then concentrated to a volume of 250 L. The resulting slurry was cooled to 5 °C and stirred for 5 h then filtered and washed with a further 80 L of ethyl acetate. The solids were dried to afford 76.9 kg of aldehyde **4** (86%).

Separation of rac-18-Nitrobenzylideneamino)phenyl)piperidine-1-carboxylate (5). Amine **17** (3.64 kg), aldehyde **4** (2.92 kg), and MTBE (24.3 kg) were charged to a vessel that was inerted and heated to 50 °C for 20 h. The batch was seeded (386 g), and heptane (27.4 kg) was charged over 1 h. The slurry was cooled to 15 °C over 1 h and filtered, washing with a premixed wash (MTBE 0.9 kg, heptane 3.3 kg) and dried by nitrogen sweep to give **5** as an off-white solid (6.04 kg, 92%).

Spectroscopic data in accord with previously published.[3a,c]

(*S*)-*tert*-Butyl 3-(4-(7-carbamoyl-2*H*-indazol-2-yl)phenyl)-piperidine-1-carboxylate 16. Imine **5** (5.84 kg), sodium azide (812 g), and DMF (44.1 kg) were charged to an inerted vessel followed by 2,6-lutidine (1.34 kg), and the batch was heated to 110 °C for 20 h. The batch was cooled to 20 °C, THF (51.7 kg) was added followed by 25 wt % $LiCl_{(aq)}$ (58 L, precooled to 5 °C), and the layers were separated. The aqueous layer was re-extracted with THF twice (2 × 36 kg). The three organics were combined and washed with 25 wt % $LiCl_{(aq)}$ (2 × 30 L). The THF solution was returned to the vessel (which had been rinsed out with water) and concentrated in vacuo (T < 35 °C) to a volume of ~60 L. It was then cooled to 20 °C, and 2 N NaOH (58.4 L) was added. The biphasic mixture was agitated and heated to 35 °C overnight. After the mixture was checked for complete ester hydrolysis, the phases were separated. Twenty-nine liters of 25 wt % $NaCl_{(aq)}$ and 29 L of 2 M HCl were added to the organic layer, and the phases were separated. The organic layer was washed with 25 wt % $NaCl_{(aq)}$ (15 L) and then distilled in vacuo to a volume of ~9 L.

Dichloromethane (46.6. kg) was added, followed by a solution of di-*tert*-butyl carbonate (3.54 kg) in DCM (~6 L) and pyridine (0.99 kg), and the batch was aged at 20−25 °C for 30 min. Ammonium bicarbonate (1.28 kg) was then added in one portion, and the batch was aged at 20−25 °C for 20 h. After checking for complete reaction, 1 M HCl (26.5 L) was added slowly, and the layers were separated. The organic phase was washed with water (13 kg) and reduced in vacuo to a volume of ~7 L. This was loaded onto ~17 kg of silica in a small oyster filter which had been prewetted with cold DCM. The filter was then eluted with DCM (~37 L) followed by a 50/50 mixture (by volume) of DCM/MTBE (~150 L). Once product had stopped coming off the silica, elution was stopped. The product-containing fractions were reduced by vacuum distillation to a volume of ~60−80 L, $T \approx 45$ °C. Seed (10 g) was then charged, and distillation continued to a volume of ~20 L. Once the product had crystallized, the slurry was cooled to 15 °C and aged for >1 h, then was filtered and washed with MTBE (5 L). The product was dried on the filter using a nitrogen sweep to give amide **16** as a tan-coloured solid (2.76 kg, 49%).

Spectroscopic data in accord with previously published reports.[3a,c]

(*S*)-2-(4-(Piperidin-3-yl)phenyl)-2*H*-indazole-7-carboxamide-Tosylate Monohydrate 1. Amide **16** (789 g) was dissolved in THF (11 L) and water (0.6 L), *p*-toluene sulphonic acid (536 g) was added, and the vessel was inerted with nitrogen. The mixture was heated to 65−67 °C and aged for 16 h to generate a tan-coloured slurry. Once the reaction had reached >99% completion, the slurry was cooled to room temperature, filtered, and washed with THF (2 L). The solid was collected and dried *in vacuo* at 40 °C to afford **1** as the tosylate monohydrate salt (797 g, 86%, >99 wt %, >99%ee) as a tan-coloured solid.

Mp = 144 °C. ^{1}H NMR (600 MHz, CD_3OD) δ 8.95 (1H, s), 8.15 (1H, dd, J = 7.1, 1.2 Hz), 8.02 (2H, m), 8.00 (1H, dd, J = 8.3, 1.2 Hz), 7.72 (2H, m), 7.49 (2H, m), 7.25 (1H, dd, J = 8.3, 7.1 Hz), 7.22 (2H, d, J = 8.0 Hz), 3.49−3.43 (2H, m), 3.16−3.04 (3H, m), 2.34 (3H, s), 2.09−2.05 (2H, m), 1.96−1.82 (2H, m). ^{13}C NMR (150.9 MHz, CD_3OD) δ 169.7, 148.1, 143.7, 143.0, 141.9, 140.5, 131.8, 130.0, 129.8, 127.3, 127.1, 125.4, 124.2, 123.3, 122.4, 50.2, 45.2, 41.1, 30.9, 24.0, 21.4.

■ ASSOCIATED CONTENT

Supporting Information. Procedures for resolution of rac-**2** via DBT salt and ee upgrade of low ee **1**. ^{1}H and ^{13}C NMR spectra for **1** tosylate salt.This material is available free of charge via the Internet at http://pubs.acs.org.

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