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Development of a Concise, Asymmetric Synthesis of a Smoothened Receptor (SMO) Inhibitor: Enzymatic Transamination of a 4-Piperidinone with Dynamic Kinetic Resolution

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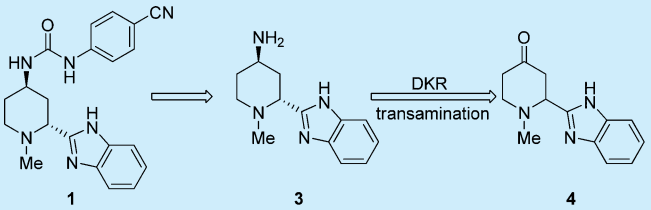
Supporting Information

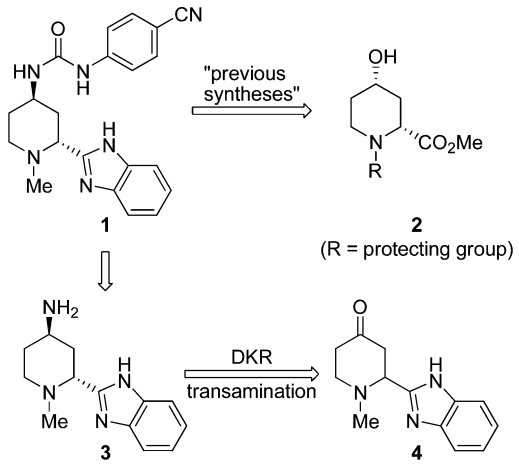
ABSTRACT: A concise, asymmetric synthesis of a smoothened receptor inhibitor (**1**) is described. The synthesis features an enzymatic transamination with concurrent dynamic kinetic resolution (DKR) of a 4-piperidone (**4**) to establish the two stereogenic centers required in a single step. This efficient reaction affords the desired *anti* amine (**3**) in >10:1 dr and >99% ee. The title compound is prepared in only five steps with 40% overall yield.



The smoothened receptor (SMO), a component of the hedgehog (Hh) signaling pathway, represents a novel therapeutic target in a broad range of human cancers.[1] Compound **1**, a potent and orally bioavailable inhibitor of SMO recently reported by Pfizer,[2] has shown early signs of efficacy and a good safety profile in phase 1 clinical trials involving patients with various blood-related cancers, including acute myeloid leukemia (AML).

To support the ongoing drug development program, a practical and scalable synthesis of **1** was required. The key synthetic challenge for the preparation of **1** was the effective and practical establishment of the *anti* stereogenic centers on the 2,4-positions of the piperidine ring. Previous syntheses[2,3] employed (2*R*,4*S*)-4-hydroxypipecolic acid derivatives **2** (Scheme 1), which were not readily available on scale. The syntheses were also tedious, and the overall yields were low.[3] Furthermore, installation of the C-4 amino group required three steps (alcohol activation, S_N2 displacement with azide, and reduction) and posed safety concerns in large scale preparation. Over the past few decades, elegant methodologies have been developed for the synthesis of chiral amines.[4] However, reports of direct enantioselective reductive amination of prochiral ketones in the absence of extra functional group interconversion steps are rare.[5] In this regard, enzymatic transamination reactions provide an advantage over traditional chemical synthesis.[6] The increased availability of transaminases and the advancement of enzyme engineering have made enzymatic transamination an increasingly attractive and viable manufacturing option for chiral amine synthesis in pharmaceuticals.[7] We envisioned that an enzymatic transamination of 4-piperidone **4** would not only generate the C-4 chiral amino group required in the penultimate **3** but also establish the *anti* C-2 stereocenter at the same time through a dynamic kinetic resolution (DKR) process.[8] It should be noted that the facile racemization and a crystallization-driven DKR of 2-aryl-4-piperidones have been reported in the literature previously.[9]

Scheme 1. Retrosynthetic Analysis



To assemble 4-piperidone **4**, we elected to explore the nucleophilic addition of benzimidazole to a pyridinium salt, which has proven to be a powerful method for the synthesis of substituted piperidines.[10] To the best of our knowledge, heterocyclic carbon nucleophiles such as benzimidazole have not been employed in this approach. We were pleased to find that the *N*-Ts benzimidazole **5**[11] was fully deprotonated with LDA at −15 °C and underwent addition to 4-methoxy *N*-methyl pyridinium triflate **6**[12] to give the corresponding dihydropyridine **7**. Following mild acidic hydrolysis of the

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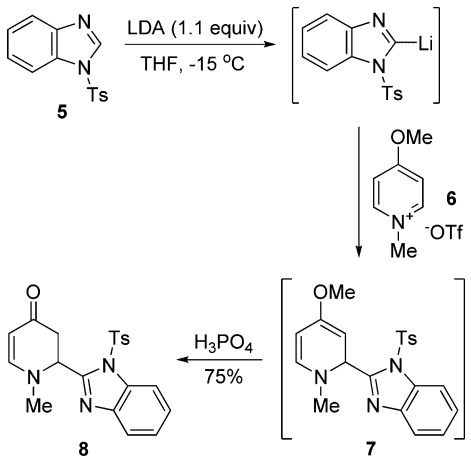
enol ether the enone could be unmasked to furnish dihydropyridone **8** (Scheme 2). A competing reaction pathway

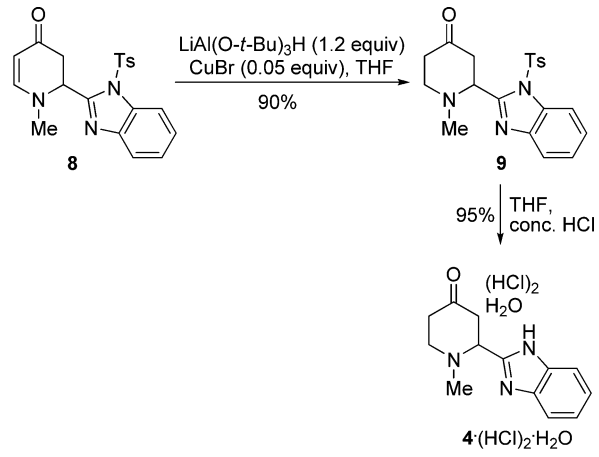
Scheme 2. Preparation of Dihydropyridone 8



was found to be the deprotonation of the α-C−H of the pyridinium salt[13] by the lithiated benzimidazole and resulted in regeneration of 10−20% of *N*-Ts benzimidazole **5**. All attempts to improve selectivity through examination of reaction parameters (bases, temperature, concentration, addition rates, etc.) were unfruitful. Nevertheless, the product could be isolated in high purity (>98%) by crystallization from isopropyl acetate in 75% yield.

Reduction of enone **8** to the corresponding ketone and removal of the Ts group were required to prepare 4-piperidone **4**. The conjugate reduction of **8** proved to be challenging due to competitive over-reduction to the saturated alcohol. Following experimentation, we found that LiAl(O-*t*-Bu)$_3$H in the presence of CuBr[14] gave excellent selectivity with less than 2% of the over-reduced alcohol observed (Scheme 3).

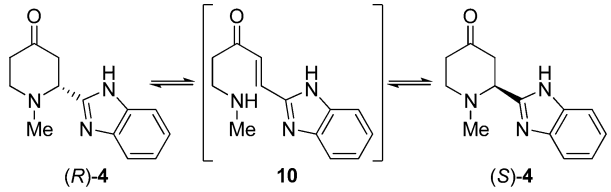
Scheme 3. Preparation of 4-Piperidone 4



Optimized conditions were identified when a slight excess of LiAl(O-*t*-Bu)$_3$H (1.2 equiv) and only 5 mol % of CuBr were employed.[15] Piperidone **9** could be isolated in 90% yield and with high purity (>99%) by crystallization from methanol−H_2O. Finally, the *N*-Ts protecting group of **9** was found to be labile to both aqueous basic (NaOH) and acidic (HCl) conditions. While the basic conditions generated over 10% of the aldol dimer byproducts, the acidic conditions (THF, conc. HCl) cleanly afforded 4-piperidone **4** in 95% isolated yield by direct filtration as a crystalline di-HCl salt hydrate.[16]

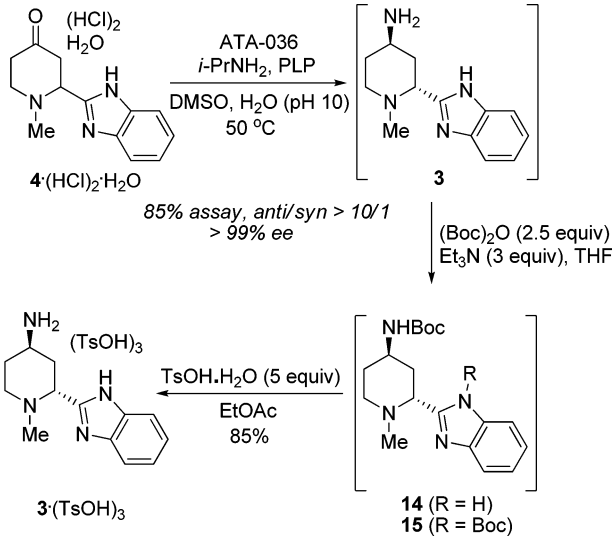
With 4-piperidone **4** in hand, the stage was set for the crucial enzymatic transamination with DKR. To investigate the feasibility of the racemization of **4**, we obtained enantiomerically pure material by chiral SFC separation of racemic **4**. Complete racemization of a single enantiomer was found to occur in less than 8 h at 40 °C in a 1/4 mixture of DMSO and aqueous pH 10 buffer, the medium for the enzymatic transamination. Although we did not have direct evidence of the corresponding ring-opened intermediate **10**, we suspected that retro-aza-Michael/aza-Michael was the mechanism by which the racemization of **4** occurred (Scheme 4).[17]

Scheme 4. Racemization of 4-Piperidone 4



Following the investigation of commercially available transaminases, we were delighted to find several enzymes that catalyzed the transamination of **4**.[18,19] Enzyme ATA-036 was identified as catalyzing highly efficient transamination with DKR to afford the desired (2*R*,4*R*)-amine **3** in high conversion and excellent selectivities. Moreover, ATA-036 demonstrated excellent thermo-stability and catalyzed the transamination of **4** in reactions with temperatures up to 75 °C. Unfortunately, 4-piperidone **4** was found to be unstable at elevated temperatures as shown by a control experiment, where in the absence of any enzyme approximately 56% of **4** decomposed after 23 h at 60 °C. Performing reactions at less than 60 °C minimized degradation of **4**, but required longer reaction times compared to reactions at higher temperatures. Consequently, a balance was obtained for the transamination reaction through heating the reaction at 50 °C for 50−60 h, generating amine **3** in 85% assay yield with an *anti*/*syn* ratio of >10:1 and >99% ee (Scheme 5).[20]

Scheme 5. Transamination and DKR of 4-Piperidone 4



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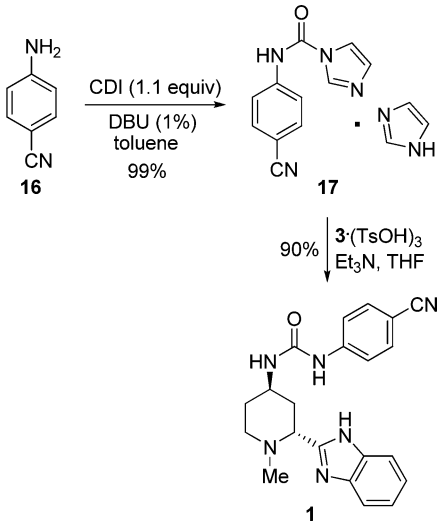
The transamination product **3** was found to be highly soluble in DMSO−H_2O and was very difficult to extract with organic solvents. Thus, we chose to employ an in situ *N*-Boc protection of the resulting amine with $(Boc)_2O$. After the transamination was complete, the DMSO−H_2O solution of **3** was treated with excess $(Boc)_2O$ and Et_3N to give a mixture of mono- and bis-Boc compounds **14** and **15**, which could be extracted with EtOAc (Scheme 5). The crude mixture of *N*-Boc compounds in EtOAc was then treated with excess tosylic acid for removal of the Boc groups. Fortunately, the tritosylate salt of **3** was directly crystallized from the reaction mixture and was isolated in 85% yield and >99% purity with the undesired *syn* isomer observed at <0.1%.

With the key penultimate **3** prepared from the enzymatic transamination, the final step of the synthesis of **1** involved formation of the urea. Previously, the urea moiety was formed by coupling of the amine with 4-cyanophenyl isocyanate. The use of this isocyanate on scale, however, is undesirable due to cost and stability issues. The corresponding *N*-carbamoylimidazole reagent, which can be prepared from the desired aniline and *N*,*N*-carbonyldiimidazole (CDI), has been utilized as an efficient alternative to the isocyanate in urea formations.[21] Indeed, we found that *N*-carbamoylimidazole **17** was easily prepared by the reaction of 4-aminobenzonitrile **16** with a slight excess of CDI (1.1 equiv) in toluene at ambient temperature, and **17** was isolated via direct filtration as a 1:1 mixture with imidazole in almost quantitative yield (Scheme 6). We also

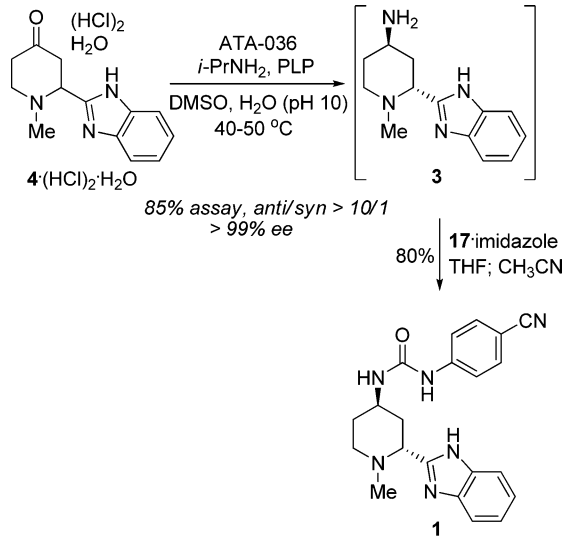
Scheme 6. Preparation of *N*-Carbamoylimidazole 17 and Synthesis of 1 with Isolated 3·$(TsOH)_3$



discovered that the addition of only 1 mol % DBU yielded shortened reaction times from over 12 h to 2−3 h.[22,23] The reaction of **17** (mixture with imidazole) with the tritosylate salt of **3** proceeded rapidly in the presence of Et_3N in THF to furnish **1** in >95% assay yield. Following aqueous workup, **1** was isolated by crystallization from acetonitrile in 90% yield and >99% purity.

Notably, we were delighted to find that *N*-carbamoylimidazole **17** reacted with **3** directly in the aqueous medium employed in the transamination reaction (Scheme 7), which eliminated the need for isolation of **3** through the *N*-Boc/de-Boc sequence.[24] A slight excess of **17** (1.2 equiv) was required to scavenge the remaining isopropylamine from the trans-

Scheme 7. Synthesis of 1 with Crude 3



amination reaction. Product **1** was formed in >90% assay yield and was isolated similarly by crystallization from acetonitrile in 80% yield and >98% purity.[25]

In summary, an efficient enzymatic transamination and DKR of 4-piperidone **4** enabled the development of a concise, asymmetric synthesis of the SMO inhibitor **1**. The entire synthesis required only five linear steps and produced **1** in 40% overall yield without the need for chromatography, thus, rendering this synthesis suitable for large scale commercial supply of **1**.

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures, compound characterization, and copies of ^1H and ^{13}C NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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(3) The current scale-up route was enabled from the original discovery route (ref 2), which produced tens of kilograms of **1** in 14 linear steps with an overall yield of only 4%. In this enabling route, (2*R*, 4*S*)-4-hydroxypipecolic acid **2** was prepared with a modified literature procedure: Gillard, J.; Abraham, A.; Anderson, P. C.;

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(11) Other protecting groups on the nitrogen of benzimidazole gave a lower yield than the tosyl group.
(12) Other pyridinium salts such as iodide, tosylate, methyl sulfate, and hexafluorophosphate were much less soluble in THF and gave poor yields.
(13) The pK_a of the α-C−H of the 4-methoxypyridinium salt was measured to be ∼33; see: Wong, F. M.; Capule, C. C.; Chen, D. X.; Gronert, S.; Wu, W. *Org. Lett.* **2008**, *10*, 2757−2760. And the D_2O quench of the reaction confirmed the deprotonation at the α-carbon of the pyridinium salt.
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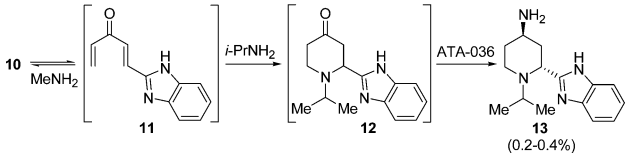
(15) Previously reported procedures required more than 1 equiv of copper (I) salts; see ref 14.
(16) Alternatively, the free base of **4** could be isolated in 87% yield with a slightly different procedure; see Supporting Information for details.
(17) Indirect evidence for the racemization mechanism came from the *N*-isopropyl impurity **13** that was formed in the transamination reaction at 0.2−0.4% level. This impurity was generated by the transamination of the *N*-isopropyl piperidone **12**, which was produced by a second retro-aza-Michael of **10** with the elimination of methylamine followed by a double aza-Michael reaction with isopropylamine, the amine donor for the transamination reaction:



(18) In the initial screening (30 °C, 19 h), eight (*R*)-selective transaminases from Codexis gave >10% assay yields with various diastereoselectivities (assay, *anti/syn* ratio): ATA-015 (15%, 1.7), ATA-016 (21%, 1.1), ATA-024 (48%, 4.1), ATA-025 (41%, 10.3), ATA-033 (42%, 9.1), ATA-034 (42%, 5.9), ATA-035 (35%, 10.2), ATA-036 (61%, 8.3). Transaminase ATA-036 was selected for further evaluation based on activity and diastereoselectivity, and subsequent chiral analysis showed that reactions with this enzyme were highly enantioselective and only produced the desired (2*R*,4*R*)-amine. Many of the Codexis (*S*)-selective transaminases also showed activity to produce the undesired (2*S*,4*S*)-amine.
(19) Both the free base and di-HCl salt of **4** were used in the transamination reaction with similar results.
(20) There were no significant differences in both diastereo- and enantioselectivity with temperatures ranging from 40 to 70 °C.
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(23) The isolated solids consisting of **17** and imidazole showed no sign of degradation after several months at ambient temperature.
(24) For urea formation with *N*-carbamoylimidzole in water, see: Padiya, K. J.; Gavade, S.; Kardile, B.; Tiwari, M.; Bajare, S.; Mane, M.; Gaware, V.; Varghese, S.; Harel, D.; Kurhade, S. *Org. Lett.* **2012**, *14*, 2814−2817.
(25) A reslurry of the isolated solids was needed to purge the undesired *syn* isomer to <1%.