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Scalable, Telescoped Hydrogenolysis–Enzymatic Decarboxylation Process for the Asymmetric Synthesis of (*R*)-α-Heteroaryl Propionic Acids

Caroline A. Blakemore,*,¶ Scott P. France,*,¶ Lacey Samp,¶ Deane M. Nason, Eddie Yang, Roger M. Howard, Karen J. Coffman, Qingyi Yang, Aaron C. Smith, Edelweiss Evrard, Wei Li, Linlin Dai, Lixia Yang, Zhiguang Chen, Qingli Zhang, Fangyan He, and Jiesen Zhang

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ABSTRACT: Enantiopure α-aryl propionic acids are useful building blocks for pharmaceutical research and can be accessed enzymatically using arylmalonate decarboxylases (AMDases) from the corresponding malonic acids. However, the intrinsic instability of malonic acids is a major drawback to this approach in which spontaneous decarboxylation can occur, subsequently eroding enantioselectivity and giving rise to racemic products. This was particularly evident for a panel of N-heterocyclic propionic acids that we wished to access using the approach. Herein, we describe a process to overcome the spontaneous decarboxylation problem in which hydrogenolysis of the corresponding dibenzyl malonates was performed in a biphasic toluene–basic aqueous buffer mixture and telescoped into the subsequent AMDase step. This procedure enabled compounds to be accessed in high enantioselectivities and was successfully demonstrated on 120 g with high yield (76%) and ee (98%).

KEYWORDS: *biocatalysis, arylmalonate decarboxylase, hydrogenolysis, α-heteroaryl propionic acids*

INTRODUCTION

α-Aryl propionic acid derivatives are synthetically useful building blocks and are common fragments in drug discovery. For example, the motif is a key part of the nonsteroidal anti-inflammatory drug (NSAID) Profen class of molecules such as naproxen and ibuprofen. For the Profen family, only the *S*-enantiomer has the desired pharmacological effect,[1] and, as for any chiral fragment, much of the synthetic challenge in accessing the motif is in generating enantiopure material. A wide range of approaches have been developed to synthesize optically pure α-aryl propionic acids[2−6] including catalytic asymmetric hydrogenation of acrylic acid precursors,[7,8] as well as biocatalytic,[9−13] auxiliary-based,[14] and resolution methods.[15,16]

We were interested in synthesizing a range of heteroaryl and substituted aryl propionic acids and were particularly attracted by biocatalytic approaches to these compounds. Biocatalysis is becoming an ever more prominent enabling technology for chemical synthesis in the pharmaceutical industry due to the high chemo- and enantioselectivity that is typically offered as well as often milder reaction conditions and environmental benefits compared to traditional chemical methods.[17−19]

Of the enzymatic approaches available, arylmalonate decarboxylases (AMDases) represent a direct way to access enantiopure α-aryl propionic acids from the corresponding malonic acids with only carbon dioxide as a byproduct (Figure 1A). One of the most well studied AMDases is from *Bordetella bronchiseptica*, which was first reported by Miyamoto and Ohta[20] and subsequently investigated in terms of enzyme structure,[21,22] mechanism,[22−24] engineering,[25−27] and substrate scope.[28−30] This enzyme has high selectivity to form *R*-configured products; however, one of the key challenges in this approach is the preparation of the required malonic acid substrates which are prone to spontaneous decarboxylation to give racemic propionic acids. Therefore, it is crucial to minimize this spontaneous process to be able to generate desired product in high enantiomeric excess.

A way to address this was reported by Gaßmeyer et al., in which the malonic acid substrates were synthesized and isolated via hydrogenolysis of their dibenzylmalonate precursors to avoid saponification conditions which can promote the decarboxylation process.[13] Therefore, we initially set out to apply this methodology to access a range of (*R*)-heteroaryl and substituted aryl propionic acids, which have so far not been exemplified in the literature using the wild-type AMDase from *B. bronchiseptica*. Unfortunately, however, many of these electron-deficient aryl malonic acids were too unstable to synthesize and isolate via this approach, prompting us to investigate their stability as well as their utility for enzymatic decarboxylation. Based on this understanding, we developed a new telescoped protocol that enabled the synthesis of a range of (*R*)-α-heteroaryl propionic acids in high ee and was amenable to scale-up and demonstrated on 120 g scale.

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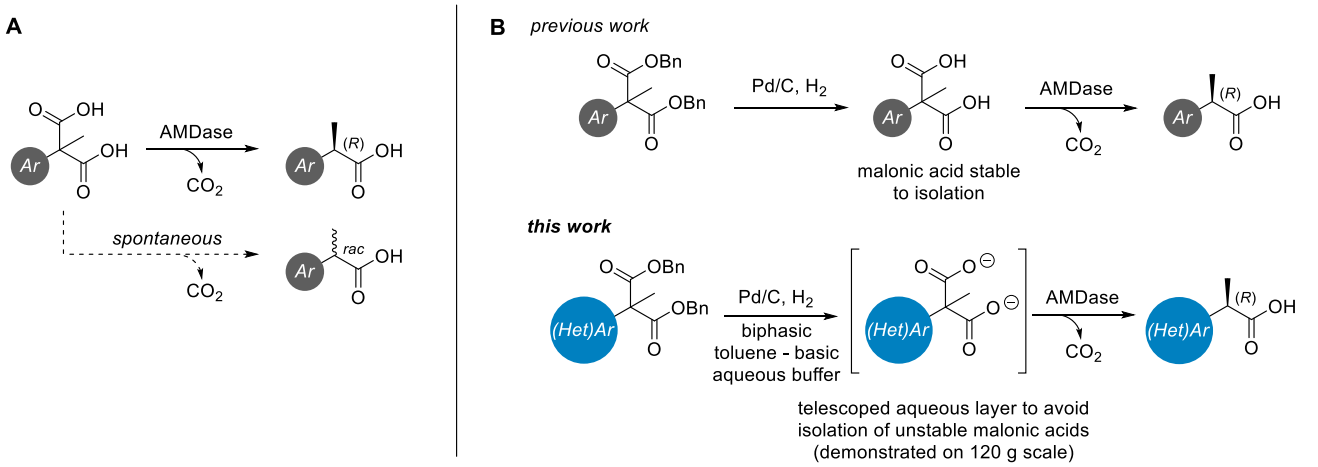

Figure 1. (A) AMDase-catalyzed enantioselective synthesis of (*R*)-*α*-aryl propionic acid derivatives. *α*-Aryl malonic acids can be prone to spontaneous decarboxylation, resulting in racemic product which lowers the overall ee achieved in the process. (B) Previous work has shown malonic acids can be synthesized by hydrogenolysis of their dibenzyl malonate precursors.[13] This work describes a telescoped, hydrogenolysis–AMDase process to (*R*)-*α*-aryl propionic acids, avoiding the isolation of unstable malonic acids, particularly when the aryl group is N-heteroaromatic.

RESULTS AND DISCUSSION

We first set out to synthesize and isolate a panel of novel heterocyclic and substituted phenyl malonic acids in order to test them as substrates for the AMDase. The synthetic route followed involved an initial cross-coupling reaction with a range of substituted (hetero)aryl halides (**1a–j**) to give dibenzyl malonate derivatives (**2a–j**). Subsequent methylation generated substrates **3a** and **3c–j** in yields of 17–58% over the two steps unoptimized and, in the case of **3b**, 70% after optimization (Figure 2). Hydrogenolysis and attempted

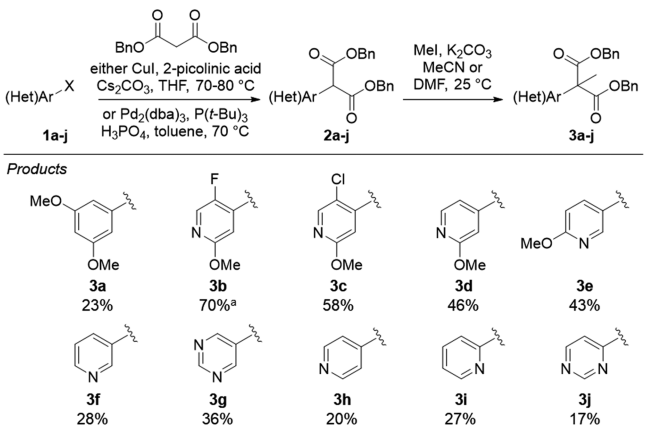

Figure 2. Synthetic route toward a panel of N-heteroaryl and substituted aryl methyl benzyl malonates **3a–j**. [a]Optimized yield over two steps from **1b**. All other quoted yields are unoptimized over two steps from **1**.

isolation of the malonic acids soon highlighted the instability of the heterocyclic compounds (**4b–j**) despite using a synthetic approach, which has previously been shown to mitigate decarboxylation for related compounds.[13] Spontaneous decarboxylation was observed during the hydrogenolysis of **3b–f** and **3h–j**, and in the case of **3g**, a complex mixture resulted. Following catalyst filtration and concentration under vacuum, either monodecarboxylation products (**5b–f**) or the bisdecarboxylation products (**6h–j**) were isolated. Only in the case of the dimethoxy phenyl ring system could the desired malonic acid **4a** be isolated cleanly as a bench-stable solid (Figure 3).

To further understand the driving force for decarboxylation, computational calculations of the ΔE values for the decarboxylation of malonic acids **4** were carried out. These suggested that the $\Delta E_{(\text{decarboxylation})}$ values for the heteroaryl compounds (**4b–j**) are all lower than those for the experimentally stable nonheteroaryl compound **4a**. Furthermore, in the case of **4i** and **4j**, negative $\Delta E_{(\text{decarboxylation})}$ values were calculated, which correlated with the experimentally observed propensity for decarboxylation of these compounds (see Supporting Information, section 7).

With these results in hand, it was apparent that the conditions were not viable for the synthesis of the heteroaromatic malonic acids. This led to an investigation of whether there were hydrogenolysis and enzymatic decarboxylation conditions under which the rate of decarboxylation could be minimized, and the isolation of the malonic acid could be avoided by employing a telescoped procedure. Choosing substrate **3b** as a model case, we tested the hydrogenolysis directly in aqueous buffer and charged AMDase enzyme to aqueous hydrogenolysis product after Pd/C catalyst filtration. Pleasingly, initial results showed that this was a promising approach with enantioenriched product observed (see Supporting Information, section 1.4).

Further understanding and optimization of the reaction parameters ultimately resulted in a telescoped process that achieved good yield and high ee of (*R*)-**5b** (Figure 4).

The key features of this procedure include the following:

(1) *Minimizing spontaneous decarboxylation with careful control of pH and temperature.* A study of malonic acid **4b** at various pH values (5–11) and temperatures (20, 40, 60 °C) showed that spontaneous decarboxylation could be effectively mitigated at pH ≥8.0 and 20 °C even after a 7 day time point (Figure 5 and Supporting Information, section 1.4.2). Therefore, the composition of the aqueous buffer was particularly important for the success of the process. pH 8.0 Tris-HCl buffer was employed so that the final AMDase step could proceed near the optimal pH for enzyme activity,[29] and crucially,

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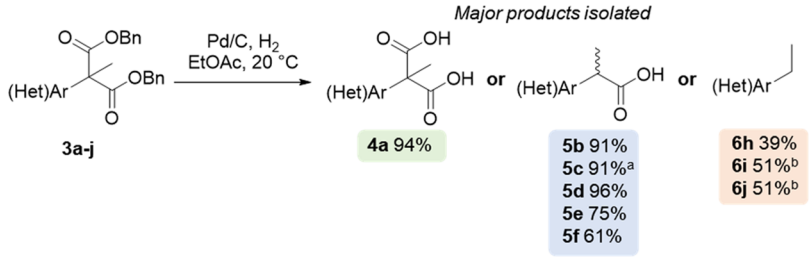

Figure 3. Hydrogenolysis of dibenzyl malonates **3a–j** in attempts to access corresponding malonic acids **4**. However, only **4a** was stable and isolable, with hydrogenolysis of **3b–j** resulting in either spontaneous decarboxylation to racemic propionic acids **5b–f**, to aryl ethanes **6h–j**, or to a complex mixture (from **3g**). [a]Pd(S)/C used instead of Pd/C. [b]Hydrogenolysis carried out at 50 °C as the reaction at 20 °C gave incomplete conversion and a complex product mixture.

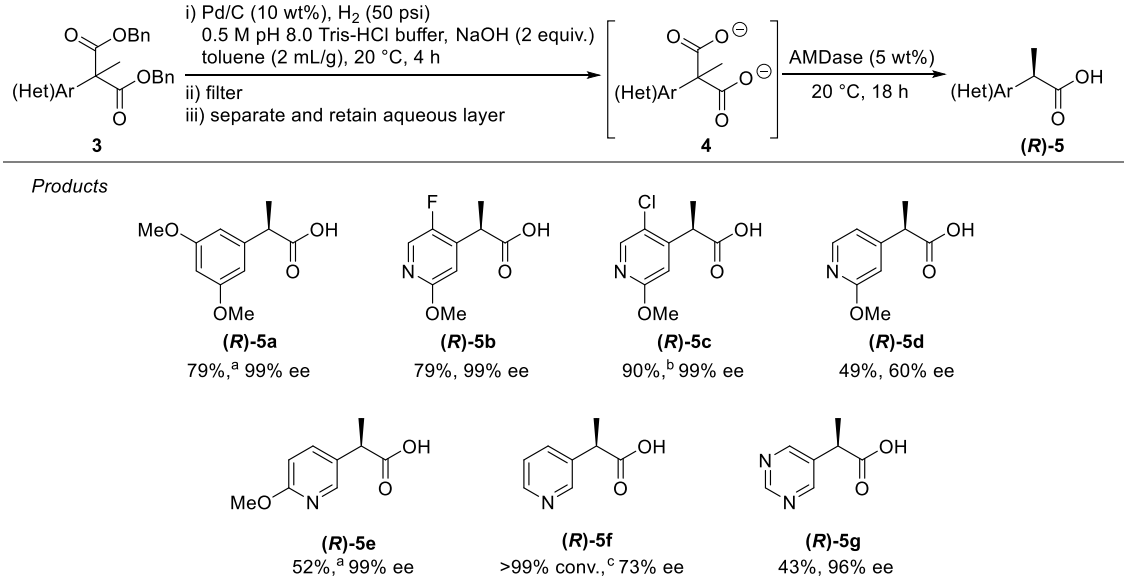

Figure 4. Telescoped hydrogenolysis–AMDase process applied to the synthesis of substituted aromatic and N-heteroaromatic (*R*)-propionic acids (*R*)-**5**. Reactions performed on 200 mg scale. [a]An additional recharge of AMDase (5 wt %) was required for full conversion. [b]Pd(S)/C used instead of Pd/C to avoid dehalogenation (see Supporting Information). [c]Unable to extract into organic layer following acidification during workup.

2 equiv of NaOH was charged before hydrogenolysis in order to neutralize the 2 equiv of carboxylic acid formed. Therefore, the initial pH of the aqueous reaction media at the start of the hydrogenolysis is pH ∼11.0 but decreases to pH 8.0 once the hydrogenolysis has gone to completion. Neutralization in this manner employs a similar concept to Lewin et al. who precipitated out α-heteroaryl-α-hydroxy malonic acids from organic solvent as their sodium salts in order to preserve them.[30]

(2) *Ensuring substrate solubility using an organic cosolvent.* The hydrogenolysis step was operated as a biphasic reaction mixture. Toluene (2 mL/g) was employed to fully dissolve substrate **3b**, which was otherwise poorly soluble in the aqueous buffer alone.

(3) *Pd/C filtration and organic cosolvent separation before the enzymatic step.* Upon completion of the hydrogenolysis, the malonate dianion **4b** resides entirely within the aqueous phase. Subsequent filtration to remove the Pd/C catalyst and separation of the toluene layer provided an aqueous buffered malonate solution. To this was simply added lyophilized, cell-free extract of the *B. bronchiseptica* AMDase, and the reaction mixture was stirred overnight at 20 °C to affect the asymmetric decarboxylation.

Pleasingly, when applied to the panel of aryl methyl dibenzyl malonates (**3a–g**), this process enabled the synthesis of (*R*)-**5a**, (*R*)-**5c**, (*R*)-**5e**, and (*R*)-**5g** in excellent ee (Figure 3). Acids (*R*)-**5d** and (*R*)-**5f** were also accessed although with modest ee due to a small degree of spontaneous decarboxylation after the hydrogenolysis step, which was not completely mitigated for these unstable compounds. The products were isolated after acidification to pH 2.5 and extraction into organic solvent, with higher isolated yields for the more lipophilic acids. In the case of (*R*)-**5f**, high water solubility precluded attempts to extract into the organic phase.

For substrate **3c**, some dechlorination was observed during the hydrogenolysis when Pd/C was used. Investigation of alternative catalysts identified Pd(S)/C as a suitable alternative in the telescoped process that completely avoided dechlorination (see Supporting Information, section 1.4.4).

Additionally, compounds **3h–j** were subjected to the telescoped process. Background decarboxylation was still observed, but interestingly, the new biphasic hydrogenolysis conditions were able to suppress the previously observed second decarboxylation event and generate racemic aryl propionic acids **5h–j** in situ. However, these compounds were not stable to acidification for subsequent isolation.

To demonstrate the scalability of the process, we performed the telescoped procedure on 50 g of **3b** with a decrease in

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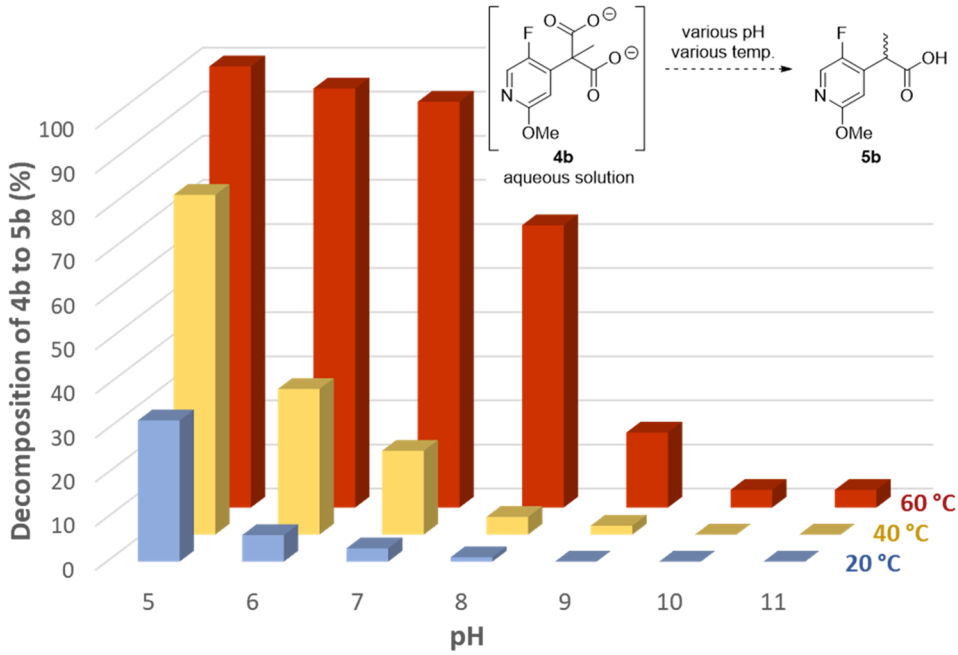
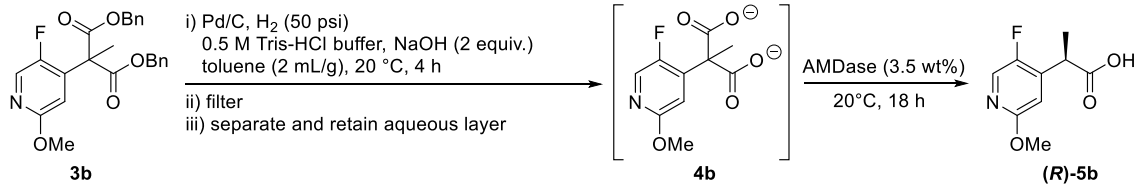


Figure 5. Stability profile of an aqueous solution of **4b** at various pH values and temperatures after 7 days showing the percentage decomposition of **4b** to **5b** from spontaneous decarboxylation (for earlier time point data, see the Supporting Information, section 1.4.2).

Table 1. Hydrogenation–AMDase Telescoped Process on Scale



entry	scale (**3b**)	Pd/C (wt %)	buffer volume (mL/g)	buffer pH	yield (%)	ee (%)
1	50 g	10	10	8.0	80	99
2	63 g	5	10	8.0	85	99
3	120 g	5	10	8.5	76	98
4	3 g	2	10	8.0	86	98
5	5 g	2	6	8.0	83	99

AMDase loading (3.5 wt %). The reaction performed equally well, generating desired (*R*)-**5b** in 80% yield and 99% ee (Table 1, entry 1).

To further improve the process, we examined the effect of lowering the Pd/C loading and buffer charges (Table 1, entries 2–5). Decreasing the loading of Pd/C was successful and aided the filtration step compared to the original 10 wt % conditions (Table 1, entries 2, 4, and 5). Using pH 8.5 Tris-HCl as the main buffer component versus pH 8.0 had no major effect on ee and validated a 0.5 pH unit acceptable range for the buffer pH (Table 1, entry 3). Finally, intensifying the reaction by reducing the volumes of buffer from 10 to 6 mL/g was also successful, with good yield and high ee achieved, enabling higher throughput (Table 1, entry 5). The largest single run was on 120 g scale, and in total, over 240 g of material was processed using this telescoped procedure.

CONCLUSIONS

A process was developed to overcome the instability of malonic acid derivatives in order to access high enantioselectivities and high yields of heteroaromatic propionic acids. Our approach utilizing a telescoped hydrogenolysis–AMDase process was suitable for large-scale application and used to process over 240 g of starting material. We hope that our addition to the asymmetric decarboxylation methodology increases its utilization through the applicability to a broader range of heterocyclic propionic acid derivatives.

EXPERIMENTAL SECTION

All reagents and solvents were obtained from commercial sources and used without further purification. Hydrogenation catalysts were purchased from Johnson Matthey (10% Pd/C catalog no. A402028-10, and 5% Pd(S)/C catalog no. A103023-5). The gene encoding the wild-type aryl malonate decarboxylase from *Bordetella bronchiseptica* (accession number Q05115)[13,20,31] was prepared in pET28a, recombinantly expressed in *Escherichia coli*, and purchased as a lyophilized cell-free extract from Prozomix, Haltwhistle, UK. This enzyme has a known strict selectivity for producing *R*-configured products.[13,28−30] See Supporting Information for further detail.

General Procedure for Telescoped Hydrogenolysis–AMDase Process on Small Scale. The processes were carried out in a parallel pressure screening reactor (Biotage Endeavor). **Hydrogenolysis**: To a solution of listed substrate

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in toluene (2 mL/g) were added 0.5 M Tris-$HCl_{(aq)}$ buffer pH as listed (10 mL/g) (containing $NaOH_{(aq)}$, 2.0 equiv relative to the listed substrate) and the listed catalyst. The resulting mixture was purged with $N_{2(g)}$ (three times) with stirring at 250 rpm and then with $H_{2(g)}$ (three times) with no stirring. The mixture was heated to 22 °C and then pressurized to the listed pressure. These conditions were held with stirring at 1000 rpm for 4 h, after which the mixture was purged with $N_{2(g)}$ and filtered (eluent, water, 10 mL/g). The filtrate layers were separated, the toluene organic phase was discarded, and the aqueous phase was analyzed by ^1H NMR (D_2O). **Enzymatic decarboxylation**: To the aqueous phase was added lyophilized AMDase powder (5 wt % relative to the dibenzyl malonate substrate or as stated), and the reaction was shaken (250 rpm) at 20 °C overnight. The pH of the reaction was *carefully* adjusted to pH 2.5 with dropwise addition of 6 M $HCl_{(aq)}$, during which off-gassing occurred. Foaming was controlled by vigorous stirring and slow pH adjustment. To this mixture was added MTBE (10 mL/g), and the mixture was filtered through diatomaceous earth (eluent, MTBE, 10 mL/g). The organic layer (containing desired compound) was separated, and the aqueous layer was extracted with MTBE (10 mL/g). The combined organic layers were dried over Na_2SO_4, filtered (eluent, MTBE 10 mL/g), and concentrated in vacuo (<35 °C bath) to give the titled compound.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.oprd.0c00397.

> Synthetic detail for the preparation of all compounds described in this paper, compound characterization, analytical methods, computational methods and data, and enzyme sequence information (DNA and protein) (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

Caroline A. Blakemore − *Medicine Design, Pfizer Inc., Groton, Connecticut 06340, United States;* orcid.org/0000-0001-7055-6379; Email: caroline.blakemore@pfizer.com

Scott P. France − *Medicine Design, Pfizer Inc., Groton, Connecticut 06340, United States;* orcid.org/0000-0003-1679-6165; Email: scott.france@pfizer.com

Authors

Lacey Samp − *Chemical Research and Development, Pfizer Inc., Groton, Connecticut 06340, United States*

Deane M. Nason − *Medicine Design, Pfizer Inc., Groton, Connecticut 06340, United States*

Eddie Yang − *Medicine Design, Pfizer Inc., Groton, Connecticut 06340, United States*

Roger M. Howard − *Medicine Design, Pfizer Inc., Groton, Connecticut 06340, United States;* orcid.org/0000-0001-5884-4896

Karen J. Coffman − *Medicine Design, Pfizer Inc., Groton, Connecticut 06340, United States*

Qingyi Yang − *Medicine Design, Pfizer Inc., Cambridge, Massachusetts 02139, United States;* orcid.org/0000-0001-5269-0494

Aaron C. Smith − *Medicine Design, Pfizer Inc., Groton, Connecticut 06340, United States;* orcid.org/0000-0001-6796-5155

Edelweiss Evrard − *Medicine Design, Pfizer Inc., Cambridge, Massachusetts 02139, United States*

Wei Li − *BioDuro LLC, Shanghai 200131, China*

Linlin Dai − *BioDuro LLC, Shanghai 200131, China*

Lixia Yang − *BioDuro LLC, Shanghai 200131, China*

Zhiguang Chen − *BioDuro LLC, Shanghai 200131, China*

Qingli Zhang − *WuXi AppTec, Shanghai 200131, China*

Fangyan He − *WuXi AppTec, Shanghai 200131, China*

Jiesen Zhang − *WuXi AppTec, Shanghai 200131, China*

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.oprd.0c00397

Author Contributions

¶C.A.B., S.P.F., and L.S. contributed equally.

Notes

The authors declare no competing financial interest.

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