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Stereoselective Synthesis of β-Branched Aromatic α-Amino Acids via Biocatalytic Dynamic Kinetic Resolution

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Abstract: β-branched noncanonical amino acids are valuable molecules in modern drug development efforts. However, they are still challenging to prepare due to the need to set multiple stereocenters in a stereoselective fashion and contemporary methods to achieve this often relies on the use of rare transition metal catalysis with designer ligand. Here, we report a biocatalytic transamination method to prepare a broad range of aromatic β-branched α-amino acids that proceeds with high diastereo- and enantioselectivity. Mechanistic studies show that the transformation proceeds through dynamic kinetic resolution that is unique to the optimal enzyme. To highlight its utility and practicality, the biocatalytic reaction is applied to the synthesis of several sp^3-rich cyclic fragments and in the first total synthesis of jomthonic acid A.

Amino acids represent one of the most indispensable and versatile building blocks in modern drug discovery.[1] In addition to its α-amino and carboxylate groups, each amino acid also contains a signature side chain that provides unique three-dimensionality and an additional structural motif for modular diversification of peptides in combinatorial synthesis. To complement the 20 canonical amino acids used in protein biosynthesis, nature also employs a variety of tailoring reactions such as hydroxylation, halogenation and methylation to further diversify these building blocks.[2] Such modification serves to modulate the physicochemical properties of the resulting noncanonical amino acid,[3] as well as the final oligopeptide which incorporates such motif (Figure 1A). Of particular note are noncanonical amino acids (ncAAs) that contain an additional stereogenic center at the β-position due to the synergistic effects of the two adjacent stereocenters to confer additional structural rigidity. For example, the presence of a β-methylphenylalanine (β-MePhe) motif in bottromycin A2 (**1a**) was found to be vital in conferring inhibitory activity towards the prokaryotic 30S ribosomal subunit as the desmethyl analogue of the natural product (**1b**) was found to be a poor antibiotic.[3a] An analogue of endomorphin bearing additional methylation at the β position of its Phe units (**3a**) was also shown to exhibit significantly improved potency and selectivity for the δ opioid receptor relative to the μ opioid receptor.[3b]

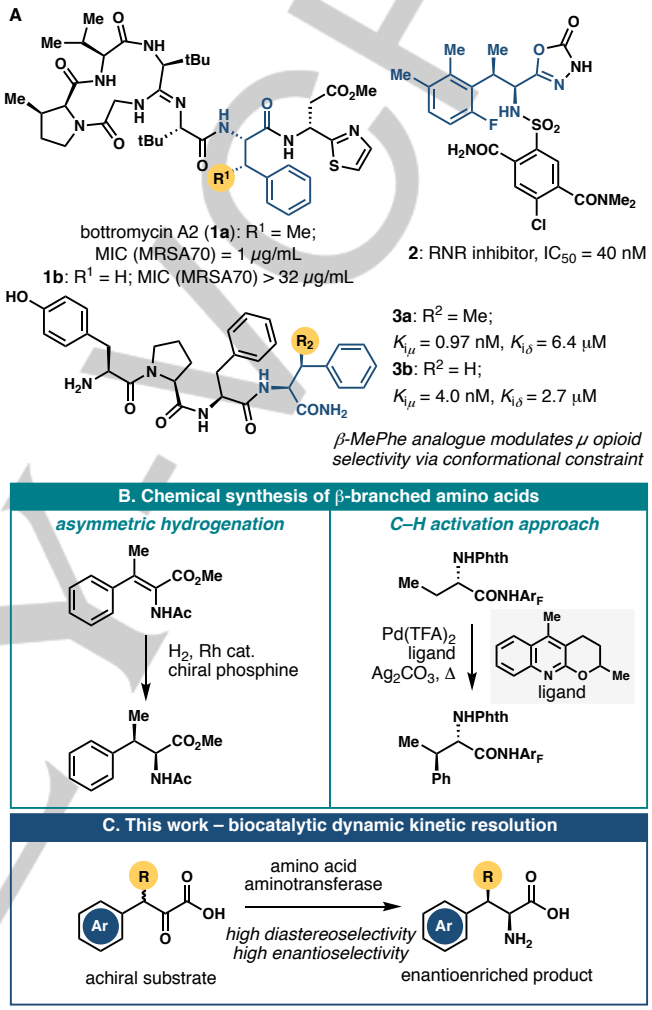

Figure 1. A. Select examples of bioactive molecules containing β-branched aromatic amino acids. **B.** Contemporary strategies to prepare β-branched aromatic amino acids via transition metal catalysis. **C.** Proposed biocatalytic synthesis of β-branched aromatic amino acids via diastereoselective transamination of α-ketoacids.

Despite their utility in modern peptide drug discovery, β-branched ncAAs remain highly challenging to synthesize due to the need to construct multiple stereocenters. Chemical synthesis of ncAAs emphasizes on the use of asymmetric transformations to set the α-stereocenter (Figure 1B) and has resulted in the development of several practical strategies, including asymmetric hydrogenation,[4] asymmetric Strecker reaction,[5] and the use of chiral auxiliaries in either polar-[6] or radical-based[7] reactions. However, synthesis of β-branched ncAAs using any of these strategies will require a separate step to establish the stereocenter at the β position. In the case of radical-based method, facile racemization at the radical center leads to a 1:1 diastereomeric mixture at the β position. Recent advances in C–H functionalization have enabled the synthesis of β-branched

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ncAAs through direct arylation or alkylation of the β-carbon.[8] However, such approaches often require the use of rare transition metal catalysts at high catalyst loading and pre-installation of a directing group and at times, suffer from sub-optimal diastereoselectivity.

Enzymatic transformations are becoming widely applied in both academia and industry.[9] By virtue of their unparalleled selectivity profile, they represent an attractive solution to the challenges associated with ncAA synthesis.[10] Indeed, a gamut of biocatalytic processes has been developed in recent years in this area. Nevertheless, only a handful of these methods allow for the generation of multiple stereocenters with high enantio- and diastereoselectivity in a single transformation. Arnold and co-workers recently reported the use of engineered tryptophan synthases for the formation of β-alkyl tryptophan analogues.[11] However, this approach is limited to indole-containing β-branched ncAAs. Similarly, Poelarends and co-workers have engineered methylaspartate ammonia lyases for the production of branched aspartate derivatives.[12] Seebeck and co-workers[13] also described the use of a self-contained enzymatic cascade for asymmetric β-methylation of α-amino acids, though this method requires the use of three enzymes in the cascade at high enzyme loading (2 mol% loading) and proceeds with low total turnover numbers overall. Gefflaut and co-workers have also reported the use of aminotransferases in the preparation of branched glutamate analogues,[14] but these reactions proceeded either via traditional kinetic resolution or with poor diastereoselectivity.

Here, we report a biocatalytic dynamic kinetic resolution (DKR) approach for the synthesis of β-branched aromatic amino acids that (1) establishes two contiguous stereocenters with complete diastereocontrol, (2) proceeds with excellent enantioselectivity towards the L-amino acid product and high catalyst efficiency, and (3) employs readily available α-ketoacid substrates (Figure 1C). Key in the reaction design is the identification of a suitable thermophilic enzyme that is able to withstand the non-physiological conditions required while also exhibiting several unique features to enable the realization of a DKR process. To the best of our knowledge, this is the first report of a biocatalytic DKR process for the production of noncanonical α-amino acids. While ω-transaminases[15] have previously been used in DKR processes including in the preparation of active pharmaceutical ingredients, applications that proceed with high diastereo- and enantioselectivity are still few and far between.

Aromatic amino acid aminotransferases (ArATs) are pyridoxal-phosphate (PLP)-dependent enzymes that are responsible for the biosynthesis of phenylalanine via transamination of phenylpyruvate with other amino acids as the amine donor. Several lines of evidence from the biosynthetic literature hint at the promiscuity of these aminotransferases. In their investigation on the biosynthetic origins of the β-MePhe moiety of mannopeptimycin,[16] Li and co-workers were able to identify a dedicated methyltransferase that methylates phenylpyruvate at the β position but were not able to find an aminotransferase within the biosynthetic gene cluster. Hypothesizing that an enzyme from primary metabolism is responsible for the latter transformation, the authors showed that TyrB, an ArAT from *E. coli*, is capable of converting β-methylphenylpyruvic acid to β-MePhe, albeit as a diastereomeric mixture at the β position. A similar observation was also made by Piel and co-workers in their biosynthetic studies on hormaomycin.[17] Importantly, these observations led us to hypothesize that ArATs are capable of accepting related pyruvate substrates that contain additional substituents at the β position to produce β-branched aromatic amino acids and that we would be able to identify a suitable ArAT that could catalyze this process with high diastereoselectivity.

Due to the importance of aromatic amino acids, ArAT is present in all domains of life and TyrB homologs have been identified from various species. Nevertheless, these ArATs have enjoyed only limited biocatalytic application. We began our investigation by examining the synthetic utility of a panel of TyrB homologs in the conversion of phenylpyruvate to β-MePhe. Of special interest are ArATs belonging to thermophilic bacteria due to the well-known benefits associated with thermostable enzymes in biocatalysis, namely the ability to withstand harsh reaction conditions, as well as superior evolvability[18] for future engineering efforts. With this feature in mind, three thermophilic enzymes, TlArAT (from *T. litoralis*),[19] PhArAT (from *P. horikoshii*)[20] and TtArAT (from *T. thermophilus*),[21] were included in our initial screening. In addition to their thermophilicity, these three enzymes have also been structurally characterized, though their use in biotechnology has not been explored before.

Our initial screening with β-methylphenylpyruvic acid (**4a**, Figure 2A) revealed that while transamination with TyrB was able to deliver the desired product in 60% yield (total turnover number, TTN = 750), it proceeded with poor diastereoselectivity (dr = 1.5:1). A similar observation was obtained in reactions with PdArAT (from *P. denitrificans*)[22] and TlArAT, whereby product **5a** was obtained only in 32% and 35% yields and moderate to poor diastereoselectivity. In contrast, reactions with PhArAT and TtArAT provided excellent diastereoselectivity for the desired product, though only moderate yields were observed. Further optimization with PhArAT improved the yield of **5a** to 56% at 60 ºC but this improvement was accompanied by a slight decrease in diastereoselectivity. In contrast, TtArAT delivered **5a** in 74% yield at 40 ºC and pH 9.0 without any loss in diastereoselectivity. Preliminary investigations also showed that TtArAT is more promiscuous than PhArAT and the former was chosen for subsequent investigation. As a benchmark, we also tested phenylalanine ammonia lyase (PAL) from *A. variabilis*[23] and phenylalanine dehydrogenases (PheDHs)[24] from *B. sphaericus* and *C. thermarum* for the preparation of **5a**, but all reactions failed to provide the desired product.

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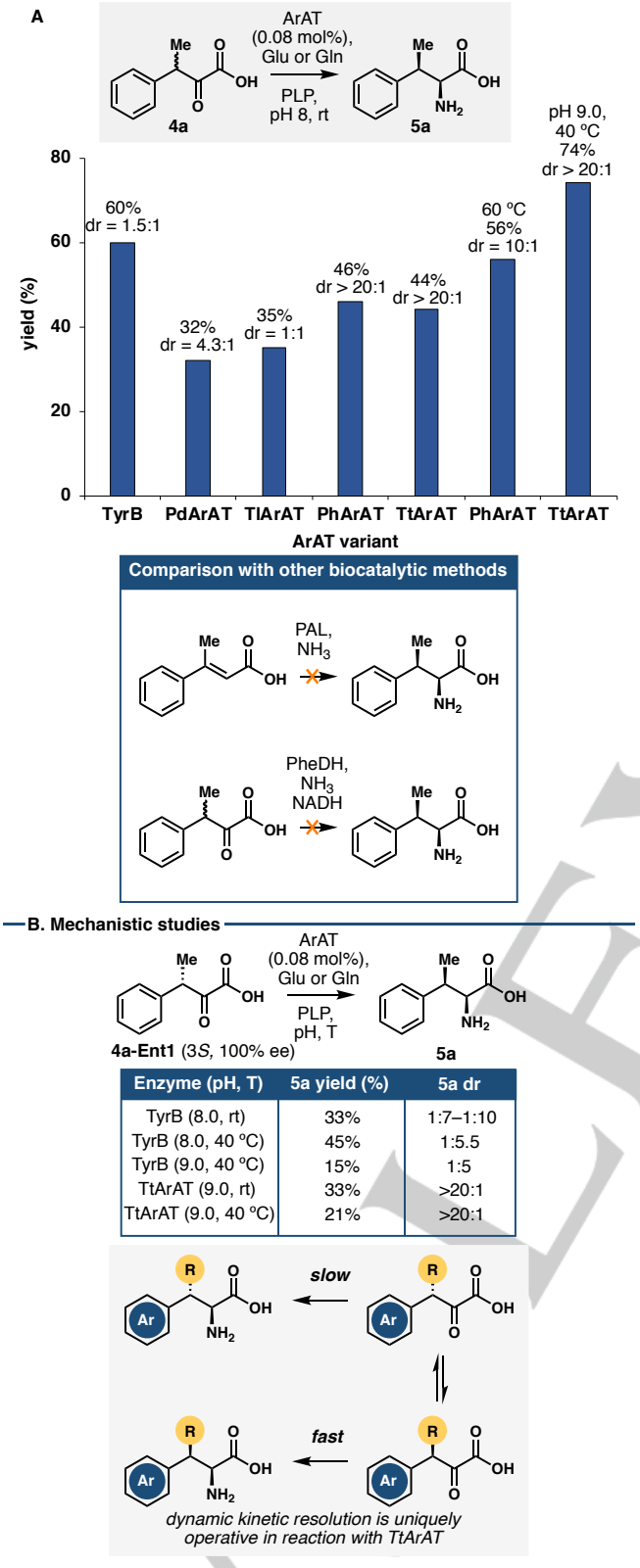

Enzyme (pH, T)	5a yield (%)	5a dr
TyrB (8.0, rt)	33%	1:7–1:10
TyrB (8.0, 40 °C)	45%	1:5.5
TyrB (9.0, 40 °C)	15%	1:5
TtArAT (9.0, rt)	33%	>20:1
TtArAT (9.0, 40 °C)	21%	>20:1

Figure 2. A. Screening of various ArATs for the transamination of **4a** and comparison with other biocatalytic methods. TyrB: L-ArAT from *E. coli*, PdArAT: L-ArAT from *P. denitrificans*, TlArAT: L-ArAT from *T. litoralis*, PhArAT: L-ArAT from *P. horikoshii*, TtArAT: L-ArAT from *T. thermophilus*, PAL: phenylalanine ammonia lyase, PheDH: phenylalanine dehydrogenase. **B.** Verification of the different types of kinetic resolution process that are operative with TyrB and TtArAT through mechanistic studies: transamination of **4a** with TtArAT proceeds through dynamic kinetic resolution featuring facile enantiomer interconversion, followed by selective transamination of one of the enantiomers.

That conversion of more than 70% could be observed with **4a** suggested that a DKR process might be operative with TtArAT. In this mechanism, the two substrate enantiomers could be rapidly interconverting but only one could preferentially undergo productive reaction with TtArAT. Prior studies in synthetic methodology, biocatalysis and mechanistic enzymology have also demonstrated that stereocenters adjacent to carbonyl groups are relatively labile under ambient conditions.[15,25] To verify this hypothesis, we conducted further mechanistic studies with TtArAT while using TyrB as a benchmark to uncover any potential mechanistic dichotomy. First, pure 3*S* and 3*R* enantiomers of **4a** (**4a-Ent1** and **4a-Ent2** respectively) were obtained via preparative chiral SFC separation and submitted to reactions with TyrB and TtArAT (Figure 2B). Transamination of **4a-Ent1** with TyrB led to only minor formation of the *syn* diastereomer **5a**, with the *anti* diastereomer formed as the major product. Additionally, the reaction became less diastereoselective at elevated temperature or pH. In contrast, TtArAT-catalyzed transamination of **4a-Ent1** proceeded with stereoinversion at C3, providing the *syn* diastereomer **5a** exclusively. As expected, all reactions with **4a-Ent2** formed *syn* **5a** as the major diastereomer regardless of the enzyme used. Using deuterium incorporation rate at the β-position of racemic **4a** in the absence of enzyme as a proxy for racemization rate, we noted that deuterium incorporation could take place at pH 8 and became more prominent at increased pH and temperature (See Supporting Information Figure S1). This observation suggested that the two substrate enantiomers are able to interconvert–albeit at different rates–under all reaction conditions tested with TyrB and TtArAT. However, TyrB shows only limited ability to discriminate between the enantiomers in its active site. While racemization of substrate enantiomers takes place more rapidly under optimal conditions with TtArAT, productive catalysis with the enzyme only takes place with the 3*R* enantiomer. Further mechanistic and structural studies to elucidate the finer details of this process are ongoing.

Following optimization, the scope and limitations of the transformation were tested on various substrates (Figure 3A). Productive reactions were observed with a variety of substrates bearing additional functional groups on their aromatic ring. In general, substitution at the *para* position on the ring (relative to the amino acid alkyl chain) is more tolerated than that at the *ortho* and *meta* positions. In several cases, the use of elevated reaction temperature (60 °C) was found advantageous in improving the reaction yields, demonstrating the benefit of employing a thermophilic enzyme in the reaction. Comparison of reaction yields obtained for products **5k**–**m**, **5o**, and **5q** suggests slight preference for substrates bearing electron withdrawing groups. However, no strong correlation between the Hammett parameters of the respective ring substituents and yields could be observed. Thus, any variation in activity likely arises primarily due to differences in steric interactions within the active site. Interestingly, increasing the size of the aryl ring to a naphthyl group led to only a small decrease in reaction yield. In cases with lower yields, non-

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enzymatic decarboxylation of the substrate was observed to be the main competing pathway.

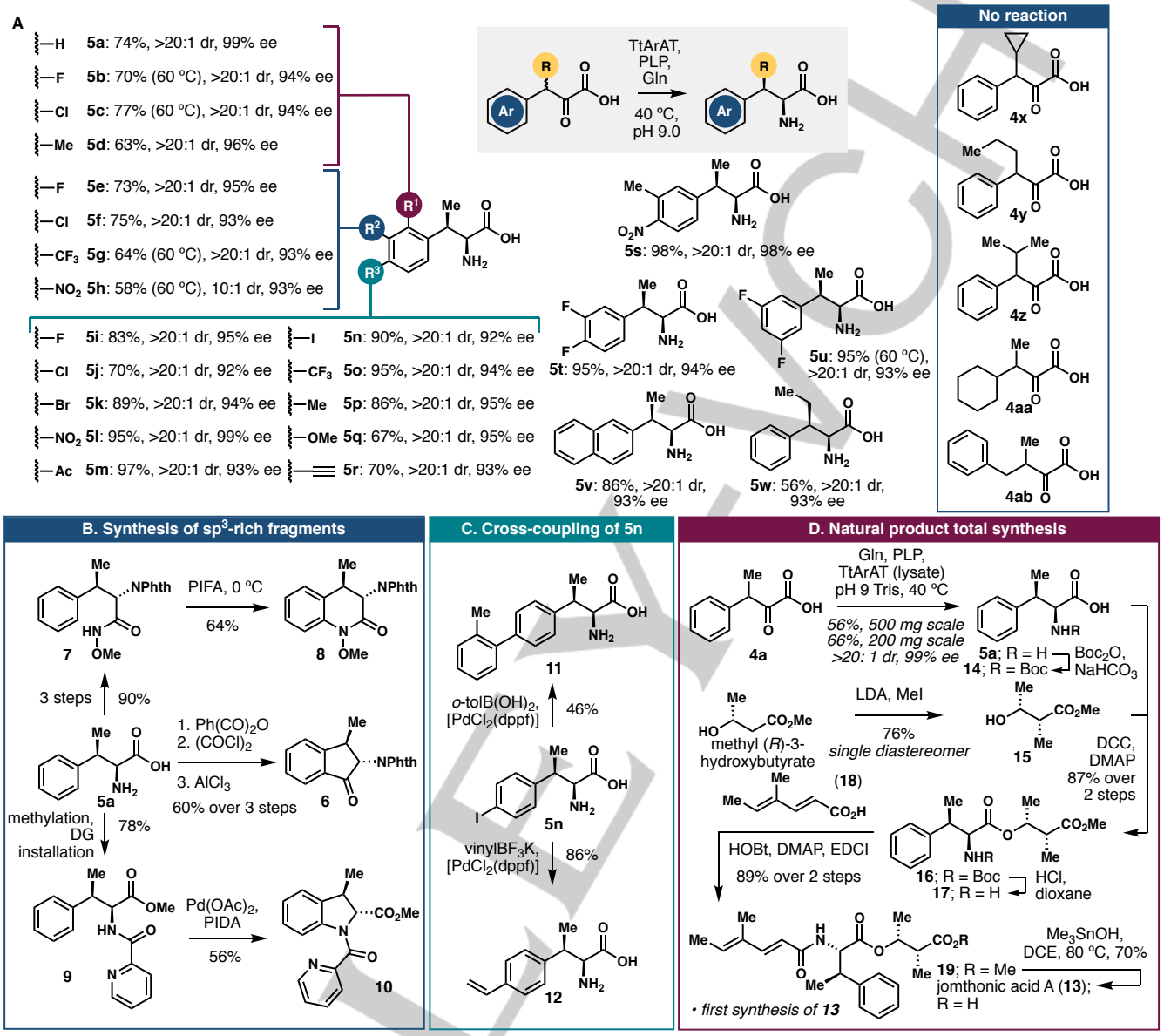

Figure 3. A. Substrate scope of biocatalytic transamination with TtArAT. Reaction conditions: α-ketoacid (10 mM, 1 equiv), Gln (30 mM, 3 equiv), PLP (0.25 mM, 2.5 mol%), TtArAT (0.008 mM, 0.08 mol%), Tris buffer (pH 9.0, 50 mM, 15 mL total volume), 24 h at 40 or 60 °C. Yields refer to isolated yields after C18 purification. See Supporting Information for details on dr and ee measurements. **B.** Derivatization of β-MePhe (**5a**) for the synthesis of several sp^3-rich fragments. **C.** Diversification of product **5n** through Pd-catalyzed Suzuki coupling. **D.** Application of biocatalytic transamination with TtArAT in the total synthesis of jomthonic acid A (**13**).

The enzymatic transformation is also well-suited for the production of β-MePhe analogues with multiple substituents (e.g. **5s**–**u**) in high yields. This feature is expected to be useful for further derivatization and manipulation of the aryl ring to arrive at more complex structures. While a small change from methyl to ethyl at the β-position is tolerated, more drastic deviations such as the introduction of a propyl, cyclopropyl and isopropyl at this position led to no reaction. This observation suggests that the active site of TtArAT is highly sensitive to steric effects at the β position of the substrate. At present, the transamination reaction is limited to the production of β-branched Phe analogues as substrates containing aliphatic chains or heteroaromatics did not participate in the reaction. Nevertheless, excellent diastereoselectivity and enantioselectivity were observed in all productive reactions. Another attractive feature of this method is the ability to attain high conversion and yield with a single enzyme system. Here, the need for L-glutamine as the amine donor with TtArAT is particularly enabling as the resulting 2-oxoglutaramate byproduct is known to undergo intramolecular cyclization and

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drive the transamination equilibrium forward.[26] In contrast, other approaches to prepare ncAA through biocatalytic reductive amination or transamination often require the use of additional enzymes for cofactor recycling, amine donor recycling or byproduct removal to drive the reaction equilibrium forward.[17]

We next sought to showcase the synthetic utility and versatility of this biocatalytic platform in the production of various sp^3-rich cyclic fragments (Figure 3B). Such complex structures are rich in three-dimensionality and are becoming increasingly valuable building blocks to "escape from flatland" in combinatorial synthesis and drug discovery.[27] Following appropriate protecting group introduction, a derivative of **5a** readily underwent a Friedel-Crafts cyclization[28] to generate an indanone product containing two stereocenters (**6**). Introduction of *N*-methoxyamide auxiliary on **5a** facilitated an oxidative cyclization in the presence of [bis(trifluoroacetoxy)iodo]benzene (PIFA)[29] to afford a multiply substituted 3,4-dihydroisoquinoline product (**8**). In a similar fashion, a chiral indoline containing two defined stereocenters (**10**) could be synthesized through the use of palladium-catalyzed C–H amination approach developed by Chen.[30] The ability to produce halogen-containing β-MePhe derivatives using this method also facilitated the synthesis of more complex products through metal-catalyzed cross-coupling (Figure 3C).[31] For example, the use of Suzuki coupling on unprotected **5n** readily afforded biaryl product **11** or styrenyl product **12**.

Finally, we demonstrated the viability and practicality of this method for preparative-scale production of β-MePhe (**5a**) to meet the material supply demands of a total synthesis campaign (Figure 3D). Here, jomthonic acid A (**13**), a soil-derived natural product with antidiabetic and antiatherogenic activities,[32] was chosen as synthetic target. Our approach commenced with the use of TtArAT-catalyzed transamination to produce **5a** on more than 500 mg scale in 56–66% yield. For subsequent synthetic manipulations, **5a** was submitted to a routine Boc protection. In parallel, alcohol **15** was prepared via a diastereoselective α-methylation of methyl (*R*)-3-hydroxybutyrate.[33] Coupling of **14** and **15** in the presence of DCC and DMAP proceeded uneventfully to afford ester **16**, which was treated with HCl in dioxane to unmask its free amine. Following peptide coupling of **17** with acid **18**, selective methyl ester hydrolysis was achieved through the use of Me_3SnOH to complete the first synthesis of jomthonic acid A.

In conclusion, by leveraging the intrinsic sequence diversity of ArATs, we identified a suitable thermophilic ArAT for the biocatalytic production of β-branched aromatic amino acids, which establishes two adjacent stereocenters with high stereoselectivity in a single transformation through a unique DKR process. The transformation is highly efficient and practical, enabling further diversification of the products obtained to generate sp^3-rich fragments for potential applications in drug discovery, as well as incorporation of the process in a chemoenzymatic synthesis. Though our substrate scope examination identified several problematic substrate classes, we envision that this issue can be addressed through further genome mining and enzyme engineering efforts. For example, the biocatalytic synthesis of all-aliphatic β-branched amino acids can potentially be achieved through the use of a similar DKR strategy with IlvE, a family of branched-chain-amino-acid aminotransferases which are responsible for the biosynthesis of leucine, isoleucine and valine.[13,34] Additionally, genome mining of other types of aminotransferases should also enable access to alternative product stereoisomers. Further studies in these areas towards the biocatalytic synthesis of more complex branched amino acids are actively being pursued in our laboratory.

Experimental Section

See Supporting Information for Experimental Details.

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Keywords: noncanonical amino acid • transaminase • biocatalysis • dynamic kinetic resolution

[1] A. Henninot, J. C. Collins, J. M. Nuss, *J. Med. Chem.* **2018**, *61*, 1382–1414.
[2] J. B. Hedges, K. S. Ryan, *Chem. Rev.* **2020**, *120*, 3161–3209.
[3] a) T. Yamada, M. Yagita, Y. Kobayashi, G. Sennari, H. Shimamura, H. Matsui, Y. Horimatsu, H. Hanaki, T. Hirose, S. Omura, T. Sunazuka, *J. Org. Chem.* **2018**, *83*, 7135–7149; b) C. Tömböly, K. E. Köver, A. Péter, D. Tourwé, D. Biyashev, S. Benyhe, A. Borsodi, M. Al-Khrasani, A. Z. Rónai, G. Tóth, *J. Med. Chem.* **2004**, *47*, 735–743; c) S. Miyahara, U. Hiroyuki, H. Shoki, O. Yoshio, WIPO Patent Application WO/2017/209155, December 7, 2017.
[4] a) M. J. Burk, M. F. Gross, J. P. Martinez, *J. Am. Chem. Soc.* **1995**, *117*, 9375–9376; b) J. Ji, C. Chen, J. Cai, X. Wang, K. Zhang, L. Shi, H. Lv, X. Zhang, *Org. Biomol. Chem.* **2015**, *13*, 7624–7627; c) G. J. Roff, R.-C. Lloyd, N. J. Turner, *J. Am. Chem. Soc.* **2004**, *126*, 4098–4099.
[5] S. J. Zuend, M. P. Coughlin, M. P. Lalonde, E. N. Jacobsen, *Nature* **2009**, *461*, 968–970.
[6] a) F. A. Davis, W. McCoull, *J. Org. Chem.* **1999**, *64*, 3396–3397; b) M. A. Beenen, D. J. Weix, J. A. Ellman, *J. Am. Chem. Soc.* **2006**, *128*, 6304–6305; c) A. Wangweerawong, J. R. Hummel, R. G. Bergman, J. A. Ellman, *J. Org. Chem.* **2016**, *81*, 1547–1557.
[7] S. Ni, A. F. Garrido-Castro, R. R. Merchant, J. N. deGruyter, D. C. Schmitt, J. J. Mousseau, G. M. Gallego, S. Yang, M. R. Collins, J. X. Qiao, K.-S. Yeung, D. R. Langley, M. A. Poss, P. M. Scola, T. Qin, P. S. Baran, *Angew. Chem. Int. Ed.* **2018**, *57*, 14560–14565; *Angew. Chem.* **2018**, *130*, 14768–14773.
[8] a) G. Chen, T. Shigenari, P. Jain, Z. Zhang, Z. Jin, J. He, S. Li, C. Mapelli, M. M. Miller, M. A. Poss, P. M. Scola, K.-S. Yeung, J.-Q. Yu, *J. Am. Chem. Soc.* **2015**, *137*, 3338–3351; b) S.-Y. Zhang, Q. Li, G. He, W. A. Nack, G. Chen, *J. Am. Chem. Soc.* **2013**, *135*, 12135–12141.
[9] a) B. Hauer, *ACS Catal.* **2020**, *10*, 8418-8427; b) S. Wu, R. Snajdrova, J.C. Moore, K. Baldenius, U.T. Bornscheuer, *Angew. Chem. Int. Ed.* **2021**, *60*, 88-119; *Angew. Chem.* **2021**, *133*, 89–123.
[10] Y.-P. Xue, C.-H. Cao, Y.-G. Zheng, *Chem. Soc. Rev.* **2018**, *47*, 1516–1561.
[11] C. E. Boville, R. A. Scheele, P. Koch, S. Brinkmann-Chen, A. R. Buller, F. H. Arnold, *Angew. Chem. Int. Ed.* **2018**, *57*, 14764–14768; *Angew. Chem.* **2018**, *130*, 14768–14773.
[12] a) M. de Villiers, V. P. Veetil, H. Raj, J. de Villiers, G. J. Poelarends, *ACS Chem. Biol.* **2012**, *7*, 1618–1628; b) H. Raj, W. Szymanski, J. de Villiers, H. J. Rozeboom, V. P. Veetil, C. R. Reis, M. de Villiers, F. J. Dekker, S. de Wildeman, W. J. Quax, A.-M. W. H. Thunnissen, B. L. Feringa, D. B. Janssen, G. J. Poelarends, *Nat. Chem.* **2012**, *4*, 478–484.
[13] C. Liao, F. P. Seebeck, *Angew. Chem. Int. Ed.* **2020**, *59*, 7184–7187; *Angew. Chem.* **2020**, *132*, 7251–7254.
[14] a) M. Xian, S. Alaux, E. Sagot, T. Gefflaut, *J. Org. Chem.* **2007**, *72*, 7560–7566; b) X. Gu, M. Xian, S. Roy-Faure, J. Bolte, D. J. Aitken, T. Gefflaut, *Tetrahedron. Lett.* **2006**, *47*, 193–196; c) S. Faure, A. A. Jensen, V. Maurat, X. Gu, E. Sagot, D. J. Aitken, J. Bolte, T. Gefflaut, L. Bunch, *J. Med. Chem.* **2006**, *49*, 6532–6538. For related work, please see: d) L. Skalden, C. Peters, J. Dickerhoff, A. Nobili, H.-J. Joosten, K. Weisz, M. Hçhne, U. T. Bornscheuer,

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ChemBioChem **2015**, *16*, 1041-1045; e) L. Skalden, C. Peters, L. Ratz, U. T. Bornscheuer, *Tetrahedron*, **2016**, *72*, 7207-7211.
[15] a) S. A Kelly, S. Pohle, S. Wharry, S. Mix, C. C. R. Allen, T. S. Moody, B. F. Gilmore, *Chem. Rev.* **2018**, *118*, 349–367; b) D. Koszelewski, D. Clay, K. Faber, W. Kroutil, *J. Mol. Cat. B: Enzym.* **2009**, *60*, 191–194; c) A. Cuetos, I. Lavandera, V. Gotor, *Chem. Commun.* **2013**, *49*, 10688–10690; d) C. K. Chung, P. G. Bulger, B. Kosjek, K. M. Belyk, N. Rivera, M. E. Scott, G. R. Humphrey, J. Limanto, D. C. Bachert, K. M. Emerson, *Org. Process. Res. Dev.* **2014**, *18*, 215–227; e) Z. Peng, J. W. Wong, E. C. Hansen, A. L. A. Puchlopek-Dermenci, H. J. Clarke, *Org. Lett.* **2014**, *16*, 860–863.
[16] Y.-T. Huang, S.-Y. Lyu, P.-H. Chuang, N.-S. Hsu, Y.-S. Li, H.-C. Chan, C.-J. Huang, Y.-C. Liu, C.-J. Wu, W.-B. Yang, T.-L. Li, *ChemBioChem* **2009**, *10*, 2480–2487.
[17] I. Hofer, M. Crusemann, M. Radzom, B. Geers, D. Flachshaar, X. Cai, A. Zeeck, J. Piel, *Chem. Biol.* **2011**, *18*, 381–391.
[18] J. D. Bloom, S. T. Labthavikul, F. H. Arnold, *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 5869–5874.
[19] G. Andreotti, M. V. Cubellis, G. Nitti, G. Sannia, X. Mai, G. Marino, M. W. W. Adams, *Eur. J. Biochem.* **1994**, *220*, 543–549.
[20] I. Matsui, E. Matsui, Y. Sakai, H. Kikuchi, Y. Kawabayasi, H. Ura, S. Kawaguchi, S. Kuramitsu, K. Harata, *J. Biol. Chem.* **2000**, *275*, 4871–4879.
[21] A. Hosono, H. Mizuguchi, H. Hayashi, M. Goto, I. Miyahara, K. Hirotsu, H. Kagamiyama, *J. Biochem.* **2003**, *134*, 843–851.
[22] A. Okamoto, Y. Nakai, H. Hayashi, K. Hirotsu, H. Kagamiyama, *J. Mol. Biol.* **1998**, *280*, 443–461.
[23] S. L. Lovelock, R. C. Lloyd, N. J. Turner, *Angew. Chem. Int. Ed.* **2014**, *53*, 4652–4656; *Angew. Chem.* **2014**, *126*, 4740–4744.
[24] a) N. Okazaki, Y. Hibino, Y. Asano, M. Ohmori, N. Numao, K. Kondo, *Gene* **1988**, *63*, 337–341; b) A. Pushpanath, E. Siirola, A. Bornadel, D. Woodlock, U. Schell, *ACS Catal.* **2017**, *7*, 3204–3209.
[25] a) S. L. Bartlett, J. S. Johnson. *Acc. Chem. Res.* **2017**, *50*, 2284–2296; b) M. Moon, S. G. Van Lanen, *Biopolymers* **2010**, *93*, 791–801.
[26] E. Heuson, F. Charmantray, J.-L. Petit, V. de Berardinis, T. Gefflaut, *Adv. Synth. Catal.* **2019**, *361*, 778–785.
[27] a) F. Lovering, J. Bikker, C. Humblet, *J. Med. Chem.* **2009**, *52*, 6752–6756; b) P. N. Devine, R. M. Howard, R. Kumar, M. P. Thompson, M. D. Truppo, N. J. Turner, *Nature. Rev. Chem.* **2018**, *2*, 409–421; c) J. I. Ramsden, S. C. Cosgrove, N. J. Turner, *Chem. Sci.* **2020**, *11*, 11104–11112.
[28] Y. Amano, K. Inoue, S. Nishiyama, *Synlett.* **2008**, *1*, 134–136.
[29] D. E. McClure, P. K. Lumma, B. H. Arison, J. H. Jones, J. J. Baldwin, *J. Org. Chem.* **1983**, *48*, 2675–2679.
[30] G. He, C. Lu, Y. Zhao, W. A. Nack, G. Chen, *Org. Lett.* **2012**, *14*, 2944–2947.
[31] a) A. D. Roy, S. Grüschow, N. Cairns, R. J. M. Goss, *J. Am. Chem. Soc.* **2010**, *132*, 12243–12245; b) W. Runguphan, S. E. O'Connor, *Org. Lett.* **2013**, *15*, 2850-1853; c) L. J. Durak, J. T. Payne, J. C. Lewis, *ACS Catal.* **2016**, *6*, 1451–1454; d) J. Latham, J.-M. Henry, H. H. Sharif, B. R. K. Menon, S. A. Shepherd, M. F. Greaney, J. Micklefield, *Nature Commun.* **2016**, *7*, 11873.
[32] Y. Igarashi, L. Yu, M. Ikeda, T. Oikawa, S. Kitani, T. Nihira, B. Bayanmunkh, W. Panbangred, *J. Nat. Prod.* **2012**, *75*, 986–990.
[33] J. S. Clark, G. Yang, A. P. Osnowski, *Org. Lett.* **2013**, *15*, 1460–1463.
[34] X. Yu, X. Wang, P. C. Engel, *FEBS J.* **2014**, *281*, 391–400.

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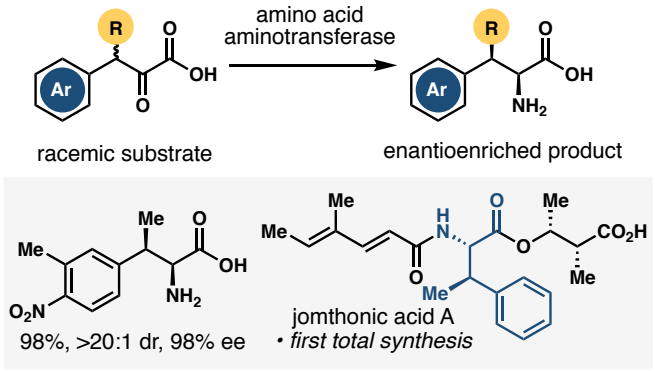

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Stereoselective Synthesis of β-Branched Aromatic α-Amino Acids via Biocatalytic Dynamic Kinetic Resolution

Dynamic Transamination: A transaminase-based dynamic kinetic resolution was developed for the preparation of β-branched aromatic α-amino acids with high diastereo- and enantioselectivity. The reaction was facilitated by the use of a thermophilic enzyme that tolerates elevated temperatures and pH and exhibits broad substrate promiscuity. The utility of the process was demonstrated in the first synthesis of jomthonic acid A.

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