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Impact of Aqueous Micellar Media on Biocatalytic Transformations Involving Transaminase (ATA); Applications to Chemoenzymatic Catalysis

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KEYWORDS: transaminase, surfactant, chemoenzymatic catalysis, biocatalysis, rivastigmine

ABSTRACT: Surfactant-enabled asymmetric ATA-catalyzed reductive aminations in aqueous buffered media are described, representative of the enhanced levels of conversion made possible by the presence of a nonionic surfactant in the water, thereby minimizing enzymatic inhibition and enabling 1-pot chemoenzymatic catalysis. Several applications are described highlighting these modified conditions that involve both biocatalysis and chemocatalysis that are environmentally responsible, indicative of the possibilities using chemistry in water. Also included herein is technology for converting a racemic benzylic alcohol to a nonracemic primary amine, and an especially efficient synthesis of the pharmaceutical (*S*)-rivastigmine.

Introduction

Few would argue with the notion that nonracemic amines play a huge role in the fine chemicals industry, as they can be found in many natural products, small molecule pharmaceuticals, and chiral auxiliaries.[1] Primary amines, in particular, are among the most important targets. Statistically, *ca.* 20% of the top 200 small molecule drugs sold in 2020 contained at least one chiral amine subunit;[2] selected examples are shown in **Figure 1**. At issue today, therefore, is no longer the question of whether incorporation of nitrogen might be needed; rather, the more focused question becomes how the amine is inserted into a selected target. Traditional routes to primary amines, of course, not only exist; they are, in fact, outstanding examples of the success of modern synthetic chemistry. However, most do not reflect the changing times, having been developed using multistep, multi-pot processes, such as formation of a protected imine followed by asymmetric hydrogenation, and then deprotection of the newly obtained chiral secondary amine.[3] Representative alternatives include transition metal-catalyzed direct asymmetric reductive amination (DARA) of carbonyl compounds with transition metal hydrides.[4,5,6] Nonetheless, while there are always exceptions, oftentimes the associated conditions tend to be harsh, waste-generating organic solvents are invariably involved, and extensive use of toxic and/or precious metals (*e.g.*, Ir, Rh, and Ru) are the norm. This latter matter can be non-trivial, as metal contamination of the desired final product(s), by FDA standards, can be a relatively common occurrence due to catalyst loadings. In the composite, such issues help explain why new technologies, including those that have led to exceptional successes based on enzymatic catalysis, are rapidly advancing. Moreover, the awarding on the Nobel Prize in 2018 in recognition of this non-petroleum-based and environmentally attractive chemistry in water could be viewed as both foreshadowing of, and encouraging developments in,

this blossoming area,[7,8,9] highlighting the role that biocatalysis is to play in the future of organic synthesis.[10,11,12]

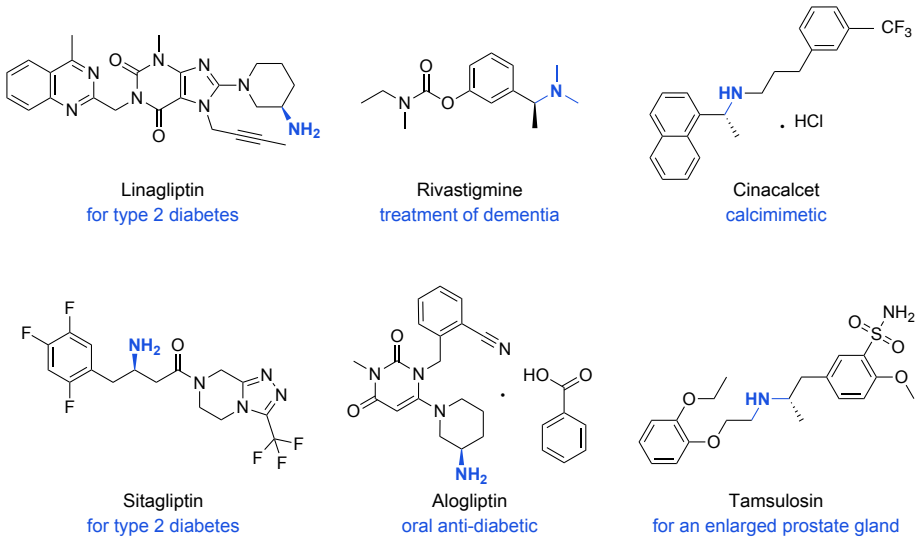


Figure 1. Selected common pharmaceuticals bearing stereogenic centers attached to nitrogen.

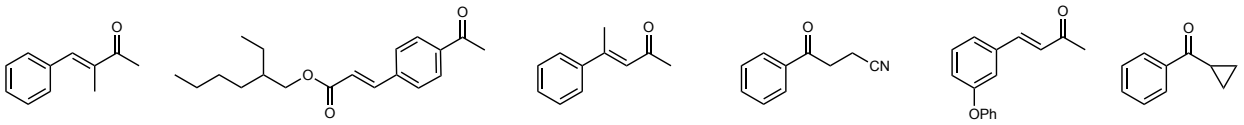
Results and discussion

In recognition of these opportunities that are increasingly available for enzymatic catalysis in aqueous media, whether utilizing naturally occurring or "new-to-nature" enzymes arrived at via directed evolution,[8] studies were initiated involving commercially available transaminases that convert ketones to nonracemic primary amines. Attention was directed towards the potentially beneficial impact, albeit unknown, that a surfactant might have simply due to its presence in the reaction medium. Prior reports from earlier studies had shown that amphiphiles in the water can lead to a "reservoir effect", providing an alternative location for water-insoluble products of enzymatic catalysis, thereby minimizing textbook enzymatic inhibition.[13,14] In an effort to document such an effect involving ATAs, which would be an especially important contribution in and of itself, we were not prepared for the multitude of atypical observations made regarding

the impact that the surfactant itself on these reductive aminations. Previously, TPGS-750-M was the surfactant of choice in all cases (*i.e.*, KREDs, EREDs, and lipases).[13,15,16] Interestingly, ATAs appear to be surfactant-dependent, suggesting that several be screened in anticipation of finding a "match" that maximizes levels of educt-to-product conversion.

This discovery was made upon analyses of six educts (**Table 1**), each treated with an ATA found (in preliminary screenings) to give the best extent of conversion in the absence of amphiphile (*i.e.*, buffer only). These bio-transformations could readily be performed on a millimole scale in a vial using a magnetic stirrer (rather than the commonly used shaker). Surfactants evaluated included TPGS-750-M, solutol, PTS-600, Brij 30, Tween 60, and Triton X-100, leading to several unprecedented observations. First, as shown for educt **1**, TPGS-750-M, solutol, Brij 30, and Triton X-100 gave similar results, with each increasing the conversion by *ca.* 20% relative to that observed in buffer alone. Each also outperformed PTS-600, and given the similarities of this amphiphile to TPGS-750-M, this was unexpected. For substrates **2, 3** and **4,** 2 wt % solutol consistently afforded the greatest extent of conversion. Likewise, for enone **5**, this same amphiphile was the most influential, albeit using 4 wt %. The behavior of cyclopropyl ketone **6** appeared to be striking in that all surfactants present in the buffered medium led to a *decrease* in the extent of conversion to its derived amine relative to buffer alone, the explanation of which remains clouded at this time.

Table 1. Screening of the aqueous reaction medium involving various ATAs.



1 2 3 4 5 6

Substrate	Enzyme	Buffer only	2 wt % TPGS-750-M/buffer	2 wt % Solutol/buffer	4 wt % Solutol/buffer	6 wt % Solutol/buffer	2 wt % PTS600/buffer	2 wt % Brij30/buffer	2 wt % Triton X-100/buffer
1	ATA-256	26	46 (56)[a]	45	48	45	13	48	44
2	ATA-256	46	52 (55)[a]	68	59	54	52	38	59
3	ATA-260	75	82	83	80	80	79	79	70
4	ATA-256	75	72	84	72	72	70	70	71
5	ATA-260	19	35	27	38	29	25	28	24
6	ATA-025	63	38	40	38	39	37	35	32

Conversions were determined by NMR. [a] TPGS-750-M (6 wt %) / triethanolamine (TEA) buffer was used.

The effect of a surfactant not only on the extent but also the kinetics of conversion of various ketones to the corresponding primary amines was investigated using six methyl ketones, each with structurally unique features. In general, as illustrated in **Figure 3**, the "boost" was, again, significant and the overall beneficial trend is clear. The highly functionalized product biaryl **8** formed from ketone **7** (**Figure 3A**) showed a 20% improvement in conversion simply due to the presence of 2 wt % TPGS-750-M in the aqueous buffered medium. In the case of *p*-CF_3-substituted acetophenone **9** (**Figure 3B**), this starting ketone reached 100% conversion to amine **10** in less than four hours, while in buffer the conversion never exceeded 89%. With enone **11** (**Figure 3C**), relatively little difference (3-5%) was seen between the buffer alone and when TPGS-750-M was present, en route to product **12**. Unexpectedly, however, Tween 60[17] increased this gap to 7–10%, maximizing at 85% conversion *vs.* 75% in buffer alone. Placement of a methyl group in the α-position of this same enone (**1**; **Figure 3D**) led to dramatically altered

catalysis, with the differential being increased to 20% for product **13** after only one hour. In this case, 2 wt % solutol[18] afforded the best outcome.

The highly lipophilic ketone **2** shown in **Figure 3E** was most receptive to the presence of additional TPGS-750-M[13] in the aqueous medium. Thus, while 2 wt % TPGS-750-M afforded an enhancement of 19% to form **14** over a 24-hour period (*i.e.*, from 22 to 41%), increasing the amount to 6 wt % resulted in furthering the extent of conversion to 56%. Interestingly, in the case of *p*-iodoacetophenone (**15**; **Figure 3F**), in buffered media alone the reaction peaks after *ca*. three hours and then begins to undergo a reversal over the 24-hour time frame from 78 to 26% conversion to amine **16**. However, in the presence of TPGS-750-M, the initial rapid conversion (80%) drops to only 70% over the same 24-hour period. This may be due to localization of the product amine within the micellar array making it unavailable for conversion back to the starting ketone, rather than undergoing reversal in the water; yet another benefit to having the surfactant simply in the medium.[19]

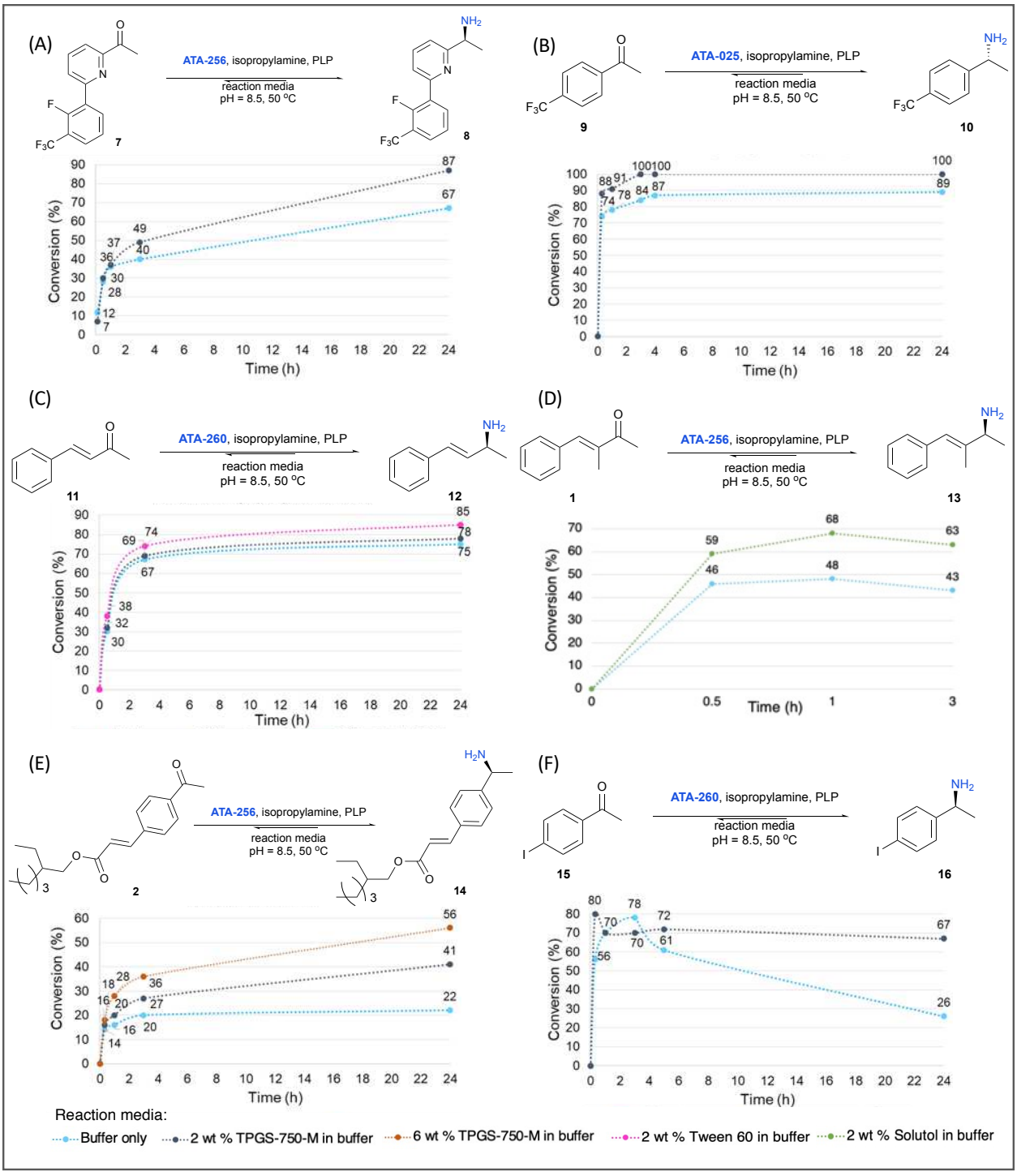


Figure 3. Time-course study of reductive aminations using an ATA. TEA buffer (0.142 M) at pH 8.5, with or without surfactant, was used as the medium. Conversions were monitored by ^{1}H NMR.

Rather than screening surfactants for every substrate, TPGS-750-M was selected as a representative amphiphile for evaluation of the scope of ketones amenable to conversion to the derived nonracemic primary amines with various ATAs in aqueous buffer under optimized conditions (**Figure 2**). Acetophenones varying in substituent and location on the ring were all readily accommodated, including highly lipophilic cases (**14** and **19**) and for one case bearing *ortho*-substitution (**20**). Cyclic ketones such as a Cbz-protected 4-oxoazepane (**21**), and *N*-Boc and *N*-Cbz-protected 3-pyrrolidinones, afforded the corresponding amines (**22** and **23**) in reasonable yields and, with the exception of **21**, high *ee*'s. Enones appeared to present no obstacles to this asymmetric reductive amination to yield allylic amines **12** and **13**. Propiophenone gave the expected nonracemic amine (**24**), as did an acetylated furan (**25**). Substituted acylpyridines (**8** and **26**) were also responsive under standard aqueous conditions at 50 °C. By contrast, as discussed recently,[20] acylated 2-bromopyridine can be converted to the same primary amine **26** under hydrogenation conditions using a Ru/BINAP catalyst in 87% yield (96% *ee*). Reductions such as these, however, require high pressures (8 atm H_2), organic solvents (THF), and high temperatures (90 °C).

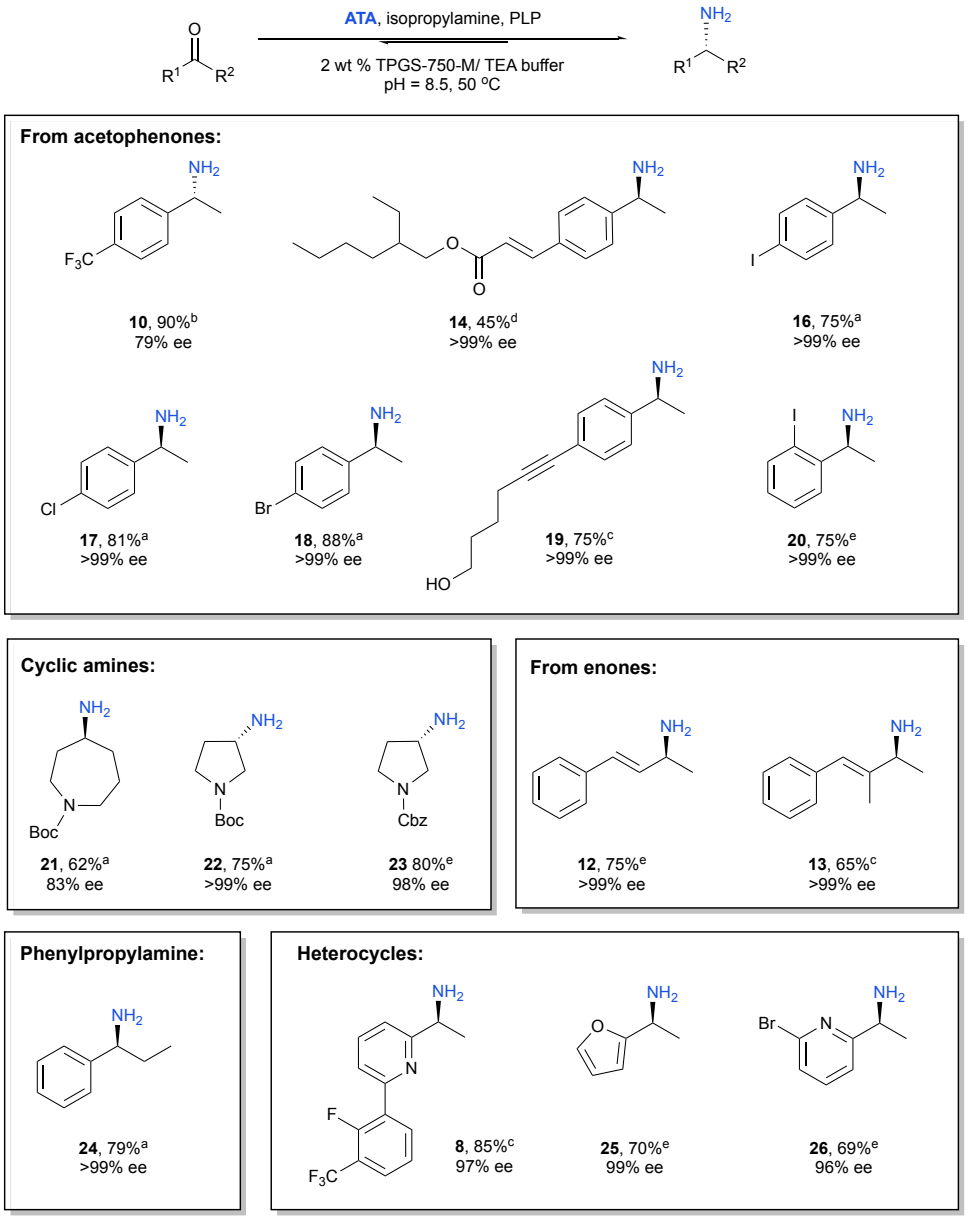


Figure 2. Substrate scope: ATA-catalyzed reductive aminations in TEA buffer (0.142 M) containing TPGS-750-M. Reactions performed with 10.0 mM ketone substrate (0.1 mmol) in a 6-dram vial equipped with a magnetic stirrer. Isolated yields are shown. PLP: pyridoxyl 5'-phosphate (co-factor). % *ee* was determined by chiral HPLC. [a]ATA-260 was used; product was isolated as a Cbz-protected (*S*)-amine; [b]ATA-025 was used; product was isolated as a Cbz-protected (*R*)-amine; [c]ATA-256 was used; product was isolated as a Cbz-protected (*S*)-amine;

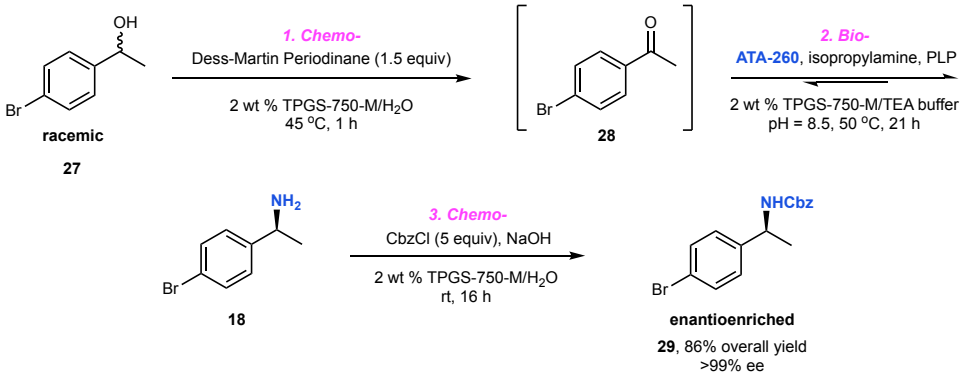
Use of 6 wt % surfactant led to the same conversion. [d]ATA-256 was used; product was isolated as an Ac-protected (*S*)-amine; [e]ATA-260 was used; product was isolated as the (*S*)-amine.

Chemoenzymatic Catalysis

The especially timely area of chemoenzymatic catalysis features both bio- and chemo-catalysis being done in the same pot.[21] Oftentimes, compartmentalization or other clever approaches are required that enable compatibility, since reagent and solvent issues may prevent both from being used in the same medium. Given that chemocatalysis in water is enabled by designer surfactants,[22,23] one solution to this fundamental problem is to do both types of chemistry in this one medium. Recent representative examples that apply this technology include the work of Hastings *et al*,[24] where TPGS-750-M-aided 1-pot cascades are composed of various classes of chemocatalysis (e.g., Mizoroki-Heck cross-coupling, ring closing metathesis, and olefin metathesis) and biocatalysis (e.g., lipase and esterase). Micklefield *et al.* also disclosed that TPGS-750-M in the aqueous medium led to a significant enhancement in the level of conversion associated with a 2-step NHase (*i.e.*, nitrile hydratase)-catalyzed reaction followed by Cu-catalyzed *N*-arylation, starting from aliphatic nitriles.[25]

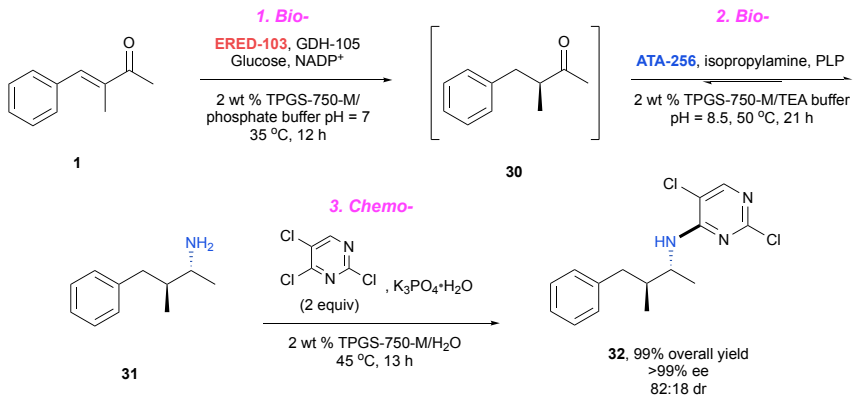
One useful application of a chemoenzymatic approach involves conversion of a racemic benzylic alcohol **27** to a nonracemic primary amine, especially where realization of either enantiomer is available. In the presence of aqueous TPGS-750-M (2 wt %), an initial Dess-Martin periodinane (DMP; 1.5 equiv) oxidization, previously unknown *in water*, gives the corresponding ketone **28** (**Scheme 1**). Without isolation, ATA-catalyzed conversion (using triethanolamine as buffer) afforded enantiopure (*S*)-amine **18**, which was subsequently converted to its *N*-Cbz derivative **29** in 86% overall yield. This transformation is equivalent to a dynamic kinetic resolution (DKR) of alcohol **27**. Choosing a different ATA that leads to the

corresponding (*R*)-isomer is also an option. This chemoenzymatic process is complimentary to that recently disclosed by Turner and co-workers using a bio-/bio-catalytic route that features use of an alcohol dehydrogenase (ADH) followed by an amine dehydrogenase (AmDH).[26] Due to the nature of the enzymes involved, however, only (*R*)-configured amines were generated using this approach. Moreover, the cost associated with co-factors such as NADH and GDH may well be another consideration.



Scheme 1. Conversion of a racemic alcohol to a nonracemic 1° amine via chemoenzymatic catalysis

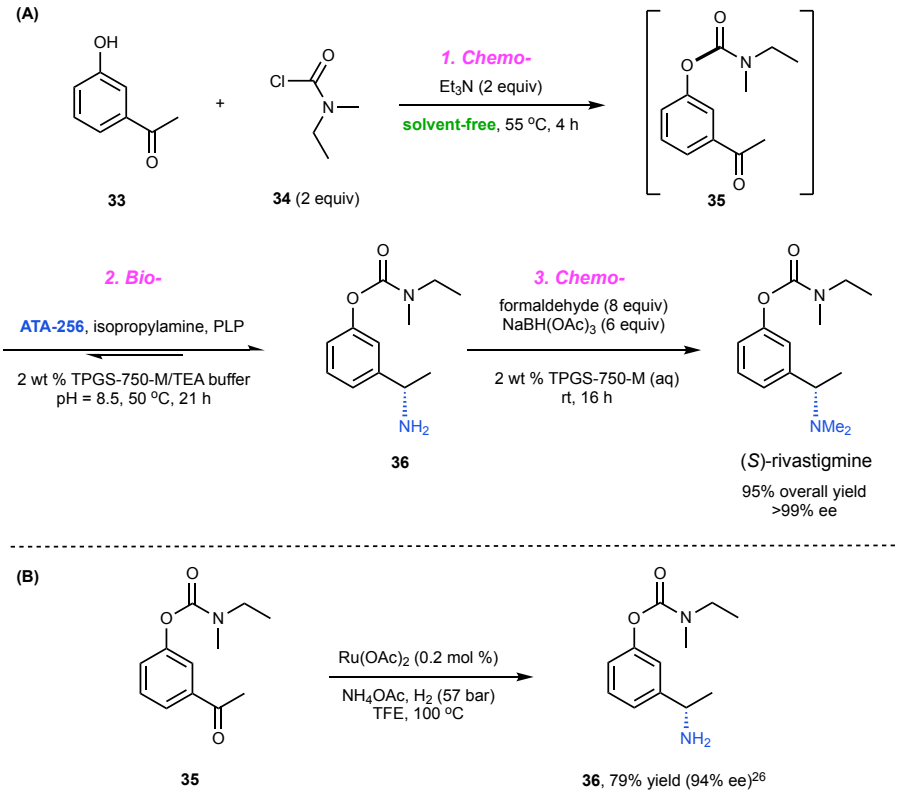
To illustrate additional synthetic options in chemoenzymatic catalysis, a 3-step tandem process in the bio-/bio-/chemo-catalysis domain was demonstrated, thereby generating two adjacent chiral centers. Initially, an ene-reductase (ERED-103) was chosen to enantioselectively reduce the double bond in enone **1**, followed by treatment with a transaminase (ATA-256) to convert the resulting ketone to the desired nonracemic amine **31**. After extraction,[27] a trichlorinated pyrimidine was introduced leading to an S_NAr reaction reflecting a very high-yielding sequence to product **32**, the major diastereomer being virtually enantiopure (**Scheme 2**).



Scheme 2. Tandem 3-step bio-, bio-, then chemo-catalysis sequence.

Lastly, an especially efficient 3-step synthesis of the widely used drug for treatment of Alzheimer's disease, (*S*)-rivastigmine, was carried out combining both chemo- and bio-catalysis (**Scheme 3A**). The sequence shown significantly simplifies several aspects of the synthesis, especially as compared with all reported approaches that involve prolonged reaction times, additional numbers of steps, and purification along the way. Prior art is summarized in **Table 2**, and illustrates the advantages of chemoenzymatic catalysis. Thus, using a clean, *solvent-free* carbamylation of 3'-hydroxyacetophenone, previously unknown, was effected to insert the required carbamate. Without isolation, TPGS-750-M (2 wt %) in aqueous buffer was added, followed by the ATA (along with the amine and co-factor PLP) leading to primary amine **36**. Newly formed intermediate **36** was then extracted with EtOAc from the aqueous reaction mixture, after which the crude material (from evaporation of the recoverable solvent) was placed again into an aqueous reaction mixture containing the surfactant, paraformaldehyde, and sodium triacetoxyborohydride to effect nitrogen dimethylation. Virtually enantiomerically pure (*S*)-rivastigmine was ultimately isolated in high overall yield. This 3-step, 2-pot synthesis represents a very mild, effective, and environmentally friendly chemoenzymatic sequence. For example, the ATA-catalyzed conversion (i.e., of **30** to **31**) can be contrasted with the recent report from Zhang

et al.[28] describing use of a nonracemically ligated Ru/C_3-TunePhos catalyst (0.2 mol %) to affect the desired reductive amination on aryl ketone **35** (**Scheme 3B**). This chemocatalytic reaction was run in trifluoroethanol (TFE) at 100 °C at 57 bar with hydrogen over 24 hours in the presence of a source of ammonia to arrive at nonracemic amine **36**.



Scheme 3. (A) A sustainable and efficient synthesis of (*S*)-rivastigmine using transaminase; (B) representative literature route using a ruthenium-catalyzed asymmetric hydrogenation towards (*S*)-rivastigmine[28]

Table 2. Comparison of literature routes to (*S*)-rivastigmine.

Entry	Reference	Catalyst to generate chiral center	Number of reaction steps	Number of pots	Total reaction time	Overall yield	*ee*[a]	E Factor[b]
1	Chang *et al*[29]	iridium (1 mol %), H_2 (60 atm), Pd/C	4	4	49.3 h	82%	96%	2779
2	Che *et al*[30]	iridium (1 mol %), H_2 (30 atm)	5	4	29.5 h	64%	>99%	270
3	List *et al*[31]	Hantzsch ester, disulfonimide	5	5	177 h	78%	>99%	3268
4	Capriati *et al*[32]	ADHs in DSM 20016 whole cells	4	3	N/A	78%	98%	N/A
5	Faber *et al*[33]	ATA-114 or ATA-117	5	4	53 h	61%	>99%	6125
6	Faber *et al*[34]	PD-ω-TA	3	3	45.5 h	66%	99%	3626
7	**This work**	**ATA-256**	**3**	**2**	**35 h**	**95%**	**>99%**	**561[c]**

[a]Represents the % *ee* of (*S*)-rivastigmine. [b]Mass of organic solvents used in the reaction and workup divided by the mass of product. [c]The unusually high E Factor is attributed to the EtOAc needed in this particular case to extract ketone **38** from the ATA reaction mixture; recovery and recycling of this solvent was not attempted.

Conclusions

In summary, the work described herein presents several advances in the chemoenzymatic catalysis category, including:

• the first study documenting quantitatively the beneficial impact of surfactants on enzymatic inhibition associated with reactions of transaminases in water;

• the first investigation illustrative of the sensitivity of ATAs to the nature of the nanomicelles in the aqueous medium that can be used to extend the levels of conversion to nonracemic primary amines;

• the first examples of mixed chem- and bio-catalytic reactions involving an ATA where *both* types of reactions involve environmentally responsible conditions;

• the first synthesis of the drug (*S*)-rivastigmine for treatment of dementia that is not only the most efficient process to date, but is performed in a single reaction vessel in the complete absence of waste-generating organic solvents.

Additional enzymatic systems are currently being evaluated in terms of their responsiveness to nonionic surfactants, including those derived via directed evolution, and will be reported in due course.

Data availability

The following files are available free of charge.

Supplementary Information. General experimental information and protocols, NMR spectra and chiral HPLC analysis for described compounds, and additional optimization tables and references (PDF).

References

1. Xu, L.-W., Luo, J. & Lu, Y. Asymmetric Catalysis with Chiral Primary Amine-Based Organocatalysts. *Chem. Commun*. **14**, 1807–1821 (2009).

2. Top 200 Small Molecule Drugs by Sales in 2020, a poster made by Jon T. Njardarson's group, https://njardarson.lab.arizona.edu/content/top-pharmaceuticals-poster (accessed 2022-01-26).

3. Xie, J.-H., Zhu, S.-F. & Zhou, Q.-L. Transition Metal-Catalyzed Enantioselective Hydrogenation of Enamines and Imines. *Chem. Rev*. **111**, 1713–1760 (2011).

4. Reshi, N. U. D., Saptal, V. B., Beller, M. & Bera, J. K. Recent Progress in Transition-Metal-Catalyzed Asymmetric Reductive Amination. *ACS Catal*. **11**, 13809–13837 (2021).

5. Kadyrov, R. & Riermeier, T. H. Highly Enantioselective Hydrogen-Transfer Reductive Amination: Catalytic Asymmetric Synthesis of Primary Amines. *Angew. Chem. Int. Ed.* **42**, 5472–5474 (2003).

6. Yin, Q., Shi, Y., Wang, J. & Zhang, X. Direct Catalytic Asymmetric Synthesis of α-Chiral Primary Amines. *Chem. Soc. Rev*. **49**, 6141–6153 (2020).

7. Simon, R. C., Richter, N., Busto, E. & Kroutil, W. Recent Developments of Cascade Reactions Involving ω-Transaminases. *ACS Catal*. **4**, 129–143 (2014).

8. Arnold, F. H. Directed Evolution: Bringing New Chemistry to Life. *Angew. Chem. Int. Ed*. **57**, 4143–4148 (2018).

9. Hammer, S. C., Knight, A. M. & Arnold, F. H. Design and Evolution of Enzymes for Non-Natural Chemistry. *Curr. Opin. Green Sustain. Chem*. **7**, 23–30 (2017).

10. Yi, D., Bayer, T., S. Badenhorst, C. P., Wu, S., Doerr, M., Höhne, M. & Bornscheuer, U. T. Recent Trends in Biocatalysis. *Chem. Soc. Rev.* **50**, 8003–8049 (2021).

11. Mangas-Sanchez, J., Sharma, M., Cosgrove, S. C., Ramsden, J. I., Marshall, J. R., Thorpe, T. W., Palmer, R. B., Grogan, G. & Turner, N. J. Asymmetric Synthesis of Primary Amines Catalyzed by Thermotolerant Fungal Reductive Aminases. *Chem. Sci*. **11**, 5052–5057 (2020).

12. Rudroff, F., Mihovilovic, M. D., Gröger, H., Snajdrova, R., Iding, H. & Bornscheuer, U. T. *Nature Catal*. **1**, 12-22. (2018).

13. Cortes-Clerget, M., Akporji, N., Zhou, J., Gao, F., Guo, P., Parmentier, M., Gallou, F., Berthon, J.-Y. & Lipshutz, B. H. Bridging the Gap between Transition Metal- and Bio-Catalysis via Aqueous Micellar Catalysis. *Nat. Commun.* **10**, 2169 (2019).

14. Guo, F. & Berglund, P. Transaminase Biocatalysis: Optimization and Application. *Green Chem.* **19**, 333–360 (2017).

15. Akporji, N., Singhania, V., Dussart-Gautheret, J., Gallou, F. & Lipshutz, B. H. Nanomicelle-Enhanced, Asymmetric ERED-Catalyzed Reductions of Activated Olefins. Applications to 1-Pot Chemo- and Bio-Catalysis Sequences in Water. *Chem. Commun.* **57**, 11847–11850 (2021).

16. Singhania, V., Cortes-Clerget, M., Dussart-Gautheret, J., Akkachairin, B., Yu, J., Akporji, N., Gallou, F. & Lipshutz, B. H. Lipase-Catalyzed Esterification in Water Enabled by Nanomicelles. Applications to 1-Pot Multi-Step Sequences. *Chem. Sci.* **13**, 1440-1445 (2022).

17. Kamande, G. M., Baah, J., Cheng, K.-J., McAllister, T. A. & Shelford, J. A. Effects of Tween 60 and Tween 80 on Protease Activity, Thiol Group Reactivity, Protein Adsorption, and Cellulose Degradation by Rumen Microbial Enzymes. *J. Dairy Sci*. **81**, 536–542 (2000).

18. Srivastava, S., Pike, A., Williamson, B. & Fenner, K. A. Novel Method for Preventing Non-specific Binding in Equilibrium Dialysis Assays Using Solutol as an Additive. *J. Pharm. Sci.* **110**, 1421–1417 (2021).

19. Andersson, M. P., Gallou, F., Klumphu, P., Takale, B. S. & Lipshutz, B. H. Structure of Nanoparticles Derived from Designer Surfactant TPGS-750-M in Water, As Used in Organic Synthesis. *Chem. Eur. J.* **24**, 6778–6786 (2018).

20. Yamada, M., Azuma, K. & Yamano, M. Highly Enantioselective Direct Asymmetric Reductive Amination of 2-Acetyl-6-Substituted Pyridines. *Org. Lett.* **23**, 3364–3367 (2021).

21. Zumbragel, N. & Groger, H. Merging Heterocyclic Chemistry and Biocatalysis in One-Pot Processes through Compartmentalization of the Reaction Steps. *Bioengineering* **5**, 60 (2018).

22. Cortes-Clerget, M., Yu, J., Kincaid, J. R. A., Walde, P., Gallou, F. & Lipshutz, B. H. *Chem. Sci.* **12**, 4237-4266 (2021).

23. Thakore, R. R.; Iyer, K. S. & Lipshutz, B. H. *Curr. Opin. Green Sustain. Chem.* **31**, 100493 (2021).

24. Hastings, C. J., Adams, N. P., Bushi, J. & Kolb, S. J. One-Pot Chemoenzymatic Reactions in Water Enabled by Micellar Encapsulation. *Green Chem*. **22**, 6187–6193 (2020).

25. Bering, L., Craven, E. J., Sowerby Thomas, S. A., Shepherd, S. A. & Micklefield, J. Merging Enzymes with Chemocatalysis for Amide Bond Synthesis. *Nat. Commun*. ***13***, 380–390 (2022).

26. Mutti, F. G., Knaus, T., Scrutton, N. S., Breuer, M. & Turner, N. J. Conversion of Alcohols to Enantiopure Amines through Dual-Enzyme Hydrogen-Borrowing Cascades. *Science.* **349**, 1525–1529 (2015).

27. Extraction required due to highly dilute nature of the reaction medium.

28. Tan, X., Gao, S., Zeng, W., Xin, S., Yin, Q. & Zhang, X. Asymmetric Synthesis of Chiral Primary Amines by Ruthenium-Catalyzed Direct Reductive Amination of Alkyl Aryl Ketones with Ammonium Salts and Molecular H_2. *J. Am. Chem. Soc*. **140**, 2024–2027 (2018).

29. Gao, G., Du, S., Yang, Y., Lei, X., Huang, H., & Chang, M. Direct Asymmetric Reductive Amination for the Synthesis of (*S*)-Rivastigmine. *Molecules* **23**, 2207–2215 (2018).

30. Yan, P.-C., Zhu, G.-L., Xie, J.-H., Zhang, X.-D., Zhou, Q.-L., Li, Y.-Q., Shen, W.-H., Che, D.-Q. Industrial Scale-Up of Enantioselective Hydrogenation for the Asymmetric Synthesis of Rivastigmine. *Org. Process Res. Dev.* **17**, 307–312 (2013).

31. Wakchaure, V. N., Kaib, P. S. J., Leutzsch, M., List, B. Disulfonimide-Catalyzed Asymmetric Reduction of *N*-Alkyl Imines. *Angew. Chem., Int. Ed.* **54**, 11852–11856 (2015).

32. Vitale, P., Perna, F. M., Agrimi, G., Pisano, I., Mirizzi, F., Capobianco, R. V., Capriati, V. Whole-Cell Biocatalyst for Chemoenzymatic Total Synthesis of Rivastigmine. *Catalysts* **8**, 55–67 (2018).

33. Fuchs, M., Koszelewski, D., Tauber, K., Kroutil, W., Faber, K. Chemoenzymatic Asymmetric Total Synthesis of (*S*)-Rivastigmine Using ω-Transaminases. *Chem*. *Commun*. **46**, 5500–5502 (2010).

34. Fuchs, M.; Koszelewski, D.; Tauber, K.; Sattler, J.; Banko, W.; Holzer, A. K.; Pickl, M.; Kroutil, W.; Faber, K. Improved Chemoenzymatic Asymmetric Synthesis of (*S*)-Rivastigmine. *Tetrahedron*, **68**, 7691–7694 (2012)

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J.D-G and B.H.L supervised the work and conceptualized the project. J.D-G, J.Y., K.G. and G.R. contributed equally to manuscript writing, experimental design and data analysis. F.G contributed to industrial perspectives and financial support.

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