

EDGE ARTICLE

Cite this: *Chem. Sci.*, 2022, 13, 1440

All publication charges for this article have been paid for by the Royal Society of Chemistry

Lipase-catalyzed esterification in water enabled by nanomicelles. Applications to 1-pot multi-step sequences†

Vani Singhania,^a Margery Cortes-Clerget,^a Jade Dussart-Gautheret,^a Bhornrawin Akkachairin,^{ab} Julie Yu,^a Nnamdi Akporji,^a Fabrice Gallou^c and Bruce H. Lipshutz^{*,a}

Esterification in an aqueous micellar medium is catalyzed by a commercially available lipase in the absence of any co-factors. The presence of only 2 wt% designer surfactant, TPGS-750-M, assists in a 100% selective enzymatic process in which only primary alcohols participate (in a 1 : 1 ratio with carboxylic acid). An unexpected finding is also disclosed where the simple additive, PhCF₃ (1 equiv. vs. substrate), appears to significantly extend the scope of usable acid/alcohol combinations. Taken together, several chemo- and bio-catalyzed 1-pot, multi-step reactions can now be performed in water.

Received 13th October 2021
Accepted 26th December 2021

DOI: 10.1039/d1sc05660c

rsc.li/chemical-science

Introduction

Textbook and related chemical methods continue to discuss the fundamental preparation of esters from acids and alcohols, typically performed in organic solvents, that require removal of water.¹ Alternatively, biocatalytic processes, while offering mild conditions, high selectivity, and, in general, environmental friendliness, are typically used in aqueous buffered media.^{2–6} Hence, esterification *via* enzymatic catalysis in water involving lipase, esterase, *etc.*, where water is the by-product, seems counterintuitive.^{7–10} Special conditions (*e.g.*, the presence of an organic solvent, prior acid activation, membrane-bound or solid supports, *etc.*) are required to address this issue.^{11,12} Only earlier this year did a report appear, outside the food industry,^{7,13} describing an enzyme-catalyzed ester synthesis in aqueous alcoholic media.¹⁴

Given the importance of the ester linkage in so many industrial products, it seems remarkable that such a functional group has yet to be constructed *via* a straightforward biocatalytic-based esterification; that such green technology indicative of the options available to synthetic chemists in water, in the absence of any organic solvent, and in particular, dipolar aprotic choices including DMSO,¹⁵ has yet to be described. We now report that use of an economical,

commercially available lipase can, indeed, catalyze esterification in water as the reaction medium, and do so using a 1 : 1 stoichiometric ratio of acid and alcohol. Moreover, these take place in the absence of any co-factor. The key to successful levels of conversion is the presence of the designer surfactant TPGS-750-M (DL- α -tocopherol methoxypolyethylene glycol succinate) in the reaction medium.¹⁶ Even further levels of conversion can be anticipated by the presence of a simple additive (1 equiv.), as disclosed herein. Applications of this new esterification, together with chemocatalysis, leads to unprecedented tandem, 1-pot processes illustrative of the potential for organic synthesis to be conducted in sequential fashion, all done in water.

Results and discussion

Esterification reactions were tested using four commercially available lipases: those from *Candida rugosa*, *Rhizopus niveus*, *Rhizomucor miehei*, and *Burkholderia cepacia*, with *Rhizomucor miehei* providing the best results (Table 1).¹⁷ For optimization purposes, lipase-catalyzed esterification between partners valeric acid (1) and *n*-hexanol (2) was initially examined. While keeping the concentration of valeric acid at either 0.25 M or 0.50 M, varied amounts of hexanol were added at both global reaction concentrations and temperatures. At 0.25 M (entries 1–3), increasing the quantity of hexanol introduced to the reaction mixture maintained at 40 °C improved the extent of product conversion. Increasing the concentration to 0.50 M (entries 4–6), unfortunately afforded no observed improvement. However, lowering the temperature to 30 °C led to essentially full conversion, thereby allowing for use of the ideal ratio of 1 : 1 (entry 8). Neither further reduction in temperature to rt (entry 7) nor increase in reaction temperature to 50 °C (entry 9) gave

^aDepartment of Chemistry and Biochemistry, University of California, Santa Barbara, CA, 93106, USA. E-mail: lipshutz@chem.ucsb.edu

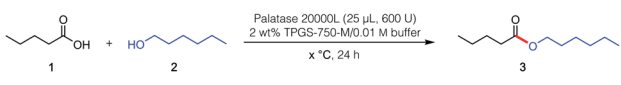
^bProgram on Chemical Biology, Chulabhorn Graduate Institute, Center of Excellence on Environmental Health and Toxicology (EHT), Ministry of Education, 54 Kamphaeng Phet 6, Laksi, Bangkok 10210, Thailand

^cNovartis Pharma AG, CH-4057 Basel, Switzerland

† Electronic supplementary information (ESI) available. See DOI: 10.1039/d1sc05660c



Table 1 Optimization of esterification in aqueous buffer + surfactant

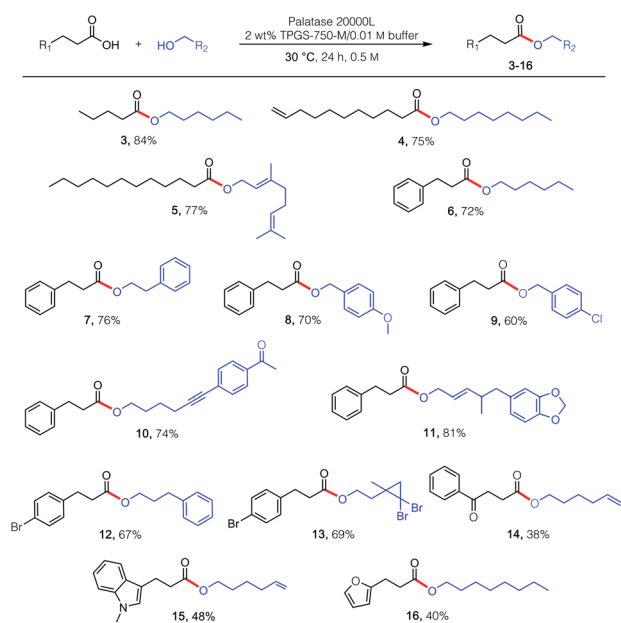


Entry	Conc. [M]	Alcohol (equiv.)	Temp. (°C)	Conversion ^a (%)
1	0.25	1	40	66
2	0.25	3	40	78
3	0.25	5	40	91
4	0.50	1	40	83
5	0.50	3	40	79
6	0.50	5	40	79
7	0.50	1	22 ^b	74
8	0.50	1	30	>99
9	0.50	1	50	9

^a Determined from crude NMR. ^b rt.

comparable levels of conversion relative to those observed at 30 °C.

Using these optimized conditions (*i.e.*, 1 : 1 acid : alcohol, 30 °C), several esterification reactions were examined, catalyzed by lipase derived from *Rhizomucor miehei* (Scheme 1; commercially available; see the ESI†). Given an enzyme's typical preference for certain structural features associated with reaction partners, as witnessed with carboxylic acids, somewhat greater flexibility was observed in terms of the alcohol that participated in the esterification process. While products 3–16 reflect these enzymatic requirements, they are solely representative of the possibilities for lipases, perhaps in general, to effect



Scheme 1 Substrate scope: lipase-catalyzed esterification in buffer under micellar conditions. Conditions: carboxylic acid (0.5 mmol; 1 equiv.), alcohol (0.5 mmol; 1 equiv.), *Rhizomucor miehei* enzyme (25 µL), 2 wt% TPGS-750-M in 0.01 M phosphate buffer solution (1 mL) at 30 °C for 24 h. Isolated yields reported.

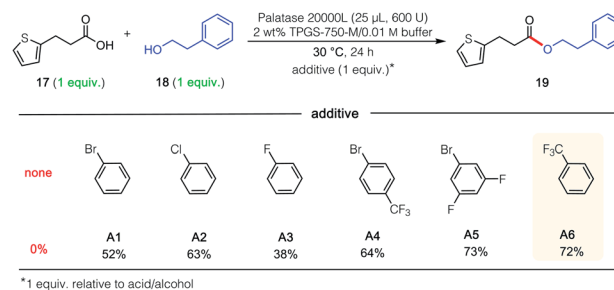
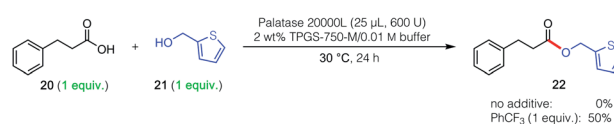


Fig. 1 Screening the effect of additives on an esterification reaction.

esterification in water. Unexpectedly, while the presence of 2 wt% TPGS-750-M (*i.e.*, 20 mg mL⁻¹ of water) in the buffered aqueous medium proved beneficial in most cases, increasing the amount to either 4 or 6 wt%, unlike that observed with KRED,¹⁸ led to no further benefit. While these esterifications in water seem unexpected, the key to success may be the presence of the micelles. Hence, the water-insoluble product esters likely locate within their inner cores, which feature a purely hydrophobic pocket. Thus, opportunities for competitive hydrolysis by water are negated due to this “reservoir” effect.¹⁸

In attempts to extend these lipase-catalyzed esterification reactions to a broader range of heteroaromatic ring-containing partners (*i.e.*, beyond those in 15 and 16; Scheme 1), educt 3-(2-thiophenyl)propionic acid (17; Fig. 1), the analog of 3-phenylpropionic acid (Scheme 1, R₁ = Ph) was examined, along with phenethyl alcohol (18), which had successfully led to product 7. In the event, and notwithstanding the thiophene's identical distance from the reactive carboxyl site, esterification was completely shut down (Fig. 1; 0% yield). Unexpectedly, therefore, when acid 17 was admixed with either the phenyl- or *p*-bromophenylpropionic acid (see 20 in Scheme 2) and the same alcohol (18), the major ester formed contained the thiophene subunit, product 19! This finding initiated a search for an additive that, ultimately, enabled esterification of 17 to 19 in 72% that was otherwise completely inhibited (notwithstanding the presence of the surfactant). This phenomenon is unlikely to be a simple solvent effect, since common solvents like methylene chloride (no conversion), hexanes (25% yield), cyclohexane (29% yield) and toluene (50% yield) were comparatively ineffective.¹⁹ Also as shown in Fig. 1, among the additives investigated (A1–A6), trifluoromethylbenzene (PhCF₃; A6) was selected given its effectiveness, commercial availability, non-chlorinated status, and attractive economics.

The unpredictable yet very intriguing impact of the additive on the inverted combination of reaction partners (*i.e.*, phenylpropanoic acid, 20 and 2-thiophenemethanol, 21) was also



Scheme 2 Impact of PhCF₃ on inverted substrate pair (*i.e.*, vs. reaction pair in Fig. 1).

Table 2 Impact of reaction variables (see reaction in Fig. 1) of additive, PhCF₃

Entry	Solvent ^a	Additive ^b (equiv.)	Yield ^c (%)
1	2 wt% TPGS-750-M/0.01 M buffer	—	0
2	2 wt% TPGS-750-M/0.01 M buffer	PhCF ₃ (0.5)	14
3	2 wt% TPGS-750-M/0.01 M buffer	PhCF₃ (1)	72
4	2 wt% TPGS-750-M/0.01 M buffer	PhCF ₃ (2)	57
5	0.01 M buffer (no surfactant)	PhCF ₃ (1)	0

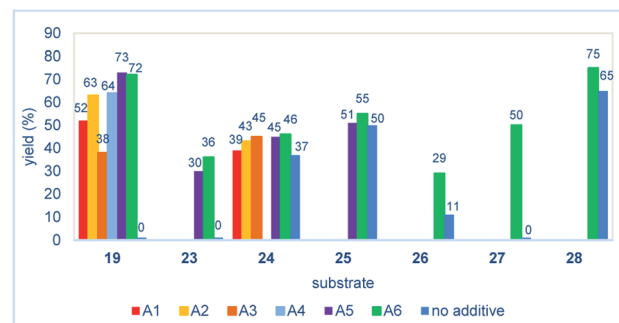
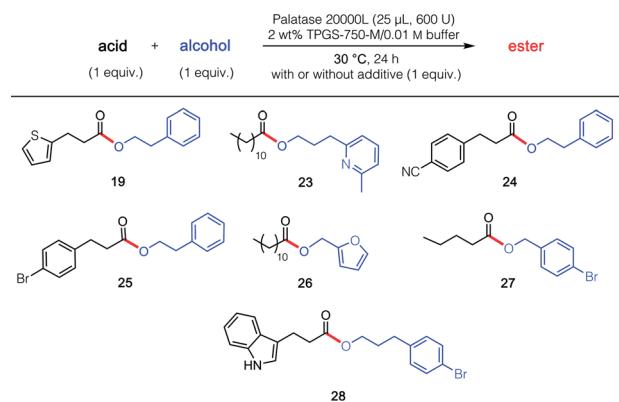
^a 1 mL of solvent used for 0.5 M global concentration. ^b One equiv. of this additive represents *ca.* 6% of the total reaction volume. ^c Isolated yield of **19**.

studied. In the event, similar results were found where, in the absence of additive, no conversion to ester **22** was observed. However, when PhCF₃ (1 equiv.) was in the aqueous reaction medium, a remarkable 50% yield was obtained (Scheme 2).

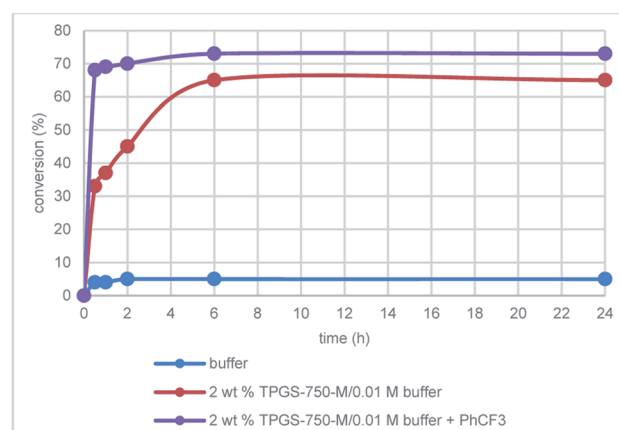
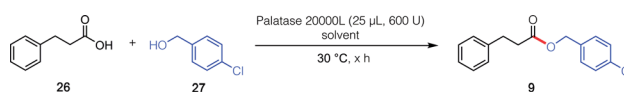
Further studies were carried out on the effect induced by this additive (PhCF₃) on the lipase derived from *Rhizomucor miehei*,²⁰ focusing on several control reactions (Table 2). The highest yield was observed in the presence of one equivalent of additive (entry 3); using half this amount dramatically lowered the extent of conversion (entry 2). Using PhCF₃ in excess (entry 4) led to a decrease in yield to 57%. Neither the micellar medium alone (entry 1) nor just the aqueous buffer (entry 5) is capable of mediating the intended reaction, a strong indication that acid **17** by itself, as observed previously (*vide supra*), is not an acceptable reaction partner for this lipase-mediated esterification.

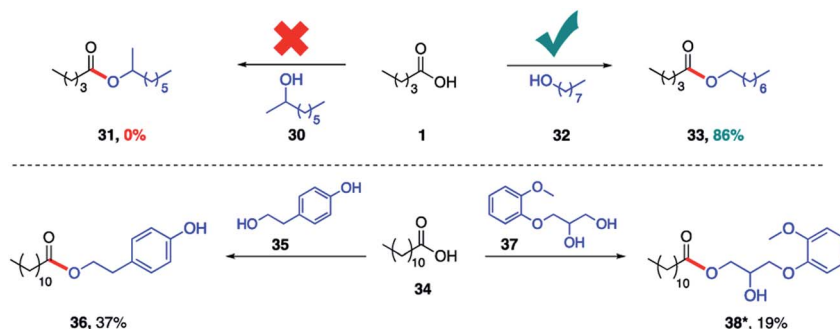
The remarkable impact of the combination of an additive **A1–A6**, together with the buffer and surfactant in the pot, was further investigated on other substrates **19**, and **23–28** (Fig. 2). Thus, in addition to the initial discovery involving esterification to product **19**, the isolated yield using PhCF₃ of esters **23** and **27** increased from 0 to 36% and from 0 to 50%, respectively. And while more modest enhancements in yields were observed for products **24–26** and **28**, the overall net positive trend using PhCF₃ is clear.

The manner in which PhCF₃ influences enzymatic esterification may be due to a positive allosteric regulation, altering the enzymatic cavity. Typically, however, this phenomenon relies on far more functionalized molecules;²¹ indeed, the overall dramatic effect mediated by such a simple additive as PhCF₃ appears to be unprecedented, perhaps suggesting that enzymatic modification leading to greater substrate tolerance may not require the types of modulators in current use. Another explanation may be the direct alteration of the entrance to the enzymatic pocket, thereby adding a variable element of “promiscuity” to the level of acceptance associated with its “natural” structural features.²² Yet another explanation is that this additive may be providing a hydrophobic layer that alters the extent of lid opening at the enzymatic site.²³ While a more definitive analysis as to which role is operative awaits further scrutiny, these observations may be indicative of future discoveries perhaps applicable to other enzymatic arrays, thereby augmenting the already huge potential of bio-catalytic processes in organic synthesis.

**Fig. 2** Impact of various additives (see Fig. 1) on lipase-catalysed esterification reactions.

Contributions by the surfactant and additive could be individually evaluated based on the “reservoir effect” first discovered in enzymatic ketone reductions (KREDs).¹⁸ This phenomenon was also observed in the lipase-catalysed esterification between 3-phenylpropanoic acid **20** and (4-chlorophenyl)methanol **29** leading to ester **9** (Fig. 3). Thus, while esterification in only buffer did not exceed 5% after a 24 hour

**Fig. 3** Impact of surfactant and additive on the representative esterification between **20** and **29**.



Scheme 3 Selective esterification of a primary alcohols catalyzed by lipase. Conditions: *Rhizomucor miehei* enzyme (25 μ L), 2 wt% TPGS-750-M/0.01 M buffer solution (1 mL) at 30 °C for 24 h. Only one product observed. Isolated yields reported. *Trace conversion in the absence of PhCF_3 .

period (reflecting commonly observed enzymatic inhibition), the presence of only 2 wt% TPGS-750-M increased the level of conversion to 65%. The same reaction with added PhCF_3 (**A6**, 1 equiv.) further raised the conversion to 73%. Noteworthy, however, was the dramatic increase in reaction rate, reaching 70% after only two hours.

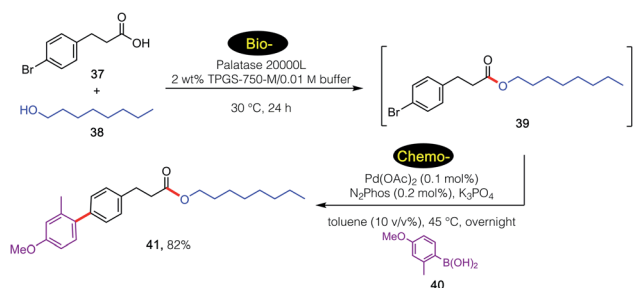
The same lipase (*Rhizomucor miehei*) was also found to selectively catalyze esterification with primary alcohols to the complete exclusion of secondary alcohols. Hence, while valeric acid (**1**) was converted in the presence of 1-octanol (**32**) to product **33** (86%), identical treatment with 2-octanol (**30**) afforded none of the corresponding ester **31** (Scheme 3). This specificity prevails even when the alcohol functional groups are present within the same molecule. Thus, irrespective of the connectivity of the alcohol on a sp^2 carbon (phenol, **35**) or an sp^3 carbon (secondary alcohol, **37**), the primary alcohol is esterified exclusively. The same selective outcome favoring esterification of a primary alcohol is observed in the presence of PhCF_3 (1 equiv.).

With water being the common denominator associated with chemo- and bio-catalysis, virtually unlimited opportunities are available for combining each in 1-pot sequences. The benefits to be realized from such a synthetic strategy include not only minimizing workups and thereby, waste creation, but also time invested (“time economy”),²⁴ and processing (“pot economy”).²⁵ Importantly, while maintaining global concentrations that typically range between 0.25 and 1 M,¹⁸ the sequence of reactions involving chemo- or bio-catalysis can be varied (*vide infra*).

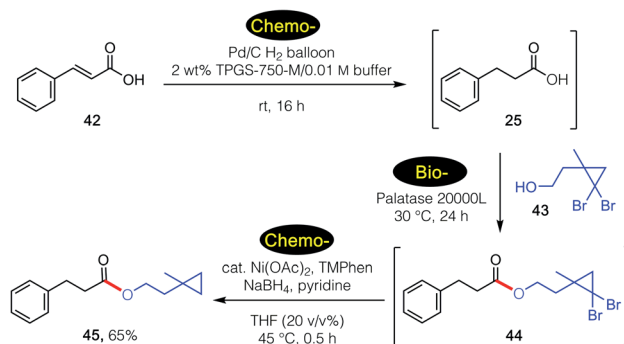
For example, a sequence involving bio- followed by chemo-catalysis is shown in Scheme 4. Lipase-catalyzed esterification of **39** with **40** leads to product **41**. Without isolation, and given the presence of the aryl bromide being amenable to a ppm Pd-catalyzed Suzuki–Miyaura coupling²⁶ involving arylboronic acid **42**, final product **43** is isolated in 82% overall yield. It is important to note the lack of competing hydrolysis of the intermediate ester after the increase of pH upon addition of base, presumably reflecting the preferred location of newly formed ester **41** within the lipophilic inner micellar cores, rather than in the basic aqueous medium.

These tandem, mixed bio- and chemo-catalysis sequences are not limited to two steps. As shown in Scheme 5, catalytic hydrogenation of cinnamic acid **44** in water in the presence of the Pd/C²⁷ led to intermediate acid **20**. Subsequent lipase-catalyzed esterification with alcohol **45** gave *gem*-dibromocyclopropane **46**, which was then reduced by nickel nanoparticles²⁸ to give product **47** in 65% overall isolated yield, all done, sequentially, in water.

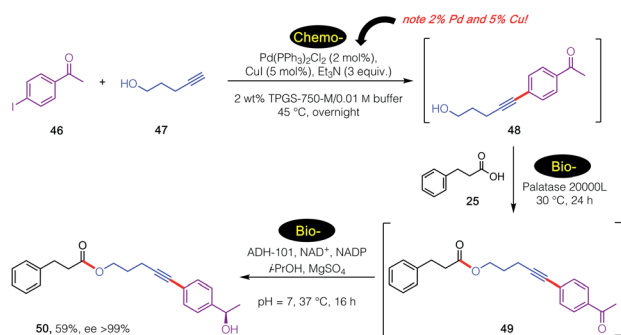
Further extending the options for this approach to chemo-enzymatic catalysis and, importantly, substantiating enzymatic compatibility, another 3-step sequence was carried out as illustrated in Scheme 6. In this case, an initial, traditional Pd-catalyzed Sonogashira coupling was performed²⁹ specifically as a test of enzymatic compatibility with such high, indeed unsustainable amounts of both Pd and Cu.³⁰ Thus, and



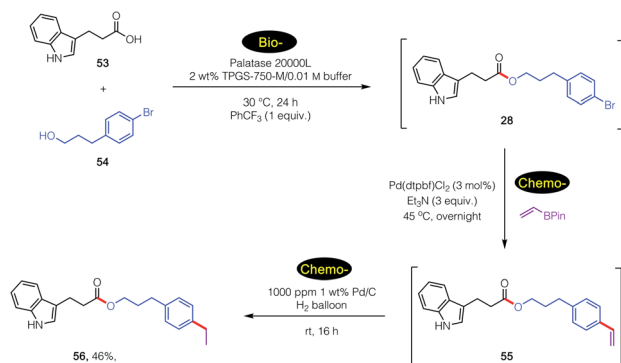
Scheme 4 A 1-pot, 2-step bio-/chemo-catalysis sequence in an aqueous micellar medium.



Scheme 5 3-Step tandem 1-pot chemo-/bio-/chemo-catalysis sequence in an aqueous micellar medium.



Scheme 6 A 1-pot, 3-step chemo-/bio-/bio-sequence in an aqueous micellar medium.



Scheme 7 A 1-pot, 3-step bio-/chemo-/chemo-sequence in an aqueous micellar medium.

notwithstanding these levels of metal present in the aqueous medium, initial product alcohol **50** was successfully subjected (after adjustment to pH = 2) to lipase-catalyzed esterification.³¹ The 1-pot reaction mixture containing ester **51** was subsequently adjusted to pH 7, after which addition of an alcohol dehydrogenase (ADH-101)¹⁸ provided secondary nonracemic alcohol **52** in 59% overall isolated yield (ee >99%).

An even more complex carboxylic acid containing an indole (**53**) was also amenable to a tandem, 1-pot process (Scheme 7). An initial biocatalytic esterification to **28**, which benefited from the presence of PhCF₃ (*vide supra*; Fig. 2), was followed (without isolation) by two consecutive chemocatalysis steps: a Pd-catalyzed Suzuki–Miyaura vinylation to **55**, and thence olefin reduction²⁷ to arrive at ester **56**. It is worthy of note that the first, selective enzymatic step takes place without protecting group chemistry (of the indole), in further alignment with the 12 Principles of Green Chemistry.^{32,33}

Conclusions

An economically attractive, commercially available lipase has been identified shown to effectively catalyze esterification reactions “in water”, requiring an ideal 1 : 1 ratio of acid and alcohol. The enzymatic process is not only tolerant of the nanomicelles present in the aqueous medium, derived from TPGS-750-M, but is made all the more effective in terms of

reaction conversion as well as avoidance of competitive hydrolysis by their presence. Also discussed is the unusual and unexpected finding that by adding PhCF₃ (1 equiv. relative to substrate) to the reaction medium the extent of conversion can be further advanced. Alteration of the enzymatic pocket using such an uncharacteristically simple additive may present exciting opportunities for extending enzymatic tolerance of additional structural features associated with a broader range of substrates of interest. Unprecedented 1-pot sequences described herein suggest that chemo- and bio-catalysis can now be used in variable combinations within the same aqueous medium, making this approach to synthesis especially attractive in terms of time and pot economy, and perhaps most noteworthy, environmental friendliness.

Author contributions

All authors contributed equally to the preparation of this ms.

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgements

This work was supported by Novartis and the NSF (CHE 18-56406).

Notes and references

- J. Otera and J. Nishikido, *Esterification: Methods, Reactions, and Applications*, Wiley-VCH, New York, 2009, DOI: 10.1002/9783527627622.
- R. O. M. A. de Souza, L. S. M. Miranda and U. T. A. Bornscheuer, *Chem.–Eur. J.*, 2017, **23**, 12040–12063.
- S. Wu, R. Snajdrova, J. C. Moore, K. Baldenius and U. T. Bornscheuer, *Angew. Chem., Int. Ed.*, 2021, **60**, 88–119.
- S. C. Hammer, A. M. Knight and F. H. Arnold, *Curr. Opin. Green Sustain. Chem.*, 2017, **7**, 23–30.
- F. H. Arnold, *Angew. Chem., Int. Ed.*, 2018, **57**, 4143–4148.
- R. A. Sheldon and J. M. Woodley, *Chem. Rev.*, 2018, **118**, 801–838.
- A. G. A. Sá, A. C. de Meneses, P. H. H. de Araújo and D. de Oliveira, *Trends Food Sci. Technol.*, 2017, **69**, 95–105.
- S. K. Karmee, *Appl. Microbiol. Biotechnol.*, 2009, **81**, 1013–1022.
- J. Müller, M. A. Sowa, B. Fredrich, H. Brundiek and U. T. Bornscheuer, *ChemBioChem*, 2015, **16**, 1791–1796.
- M. W. Larsen, D. F. Zielinska, M. Martinelle, A. Hidalgo, L. J. Jensen, U. T. Bornscheuer and K. Hult, *ChemBioChem*, 2010, **11**, 796–801.
- N. N. Gandhi, N. S. Patil, S. B. Sawant, J. B. Joshi, P. P. Wangikar and D. Mukesh, *Catal. Rev.*, 2000, **42**, 439–480.
- L. Mestrom, J. G. R. Claessen and U. Hanefeld, *ChemCatChem*, 2019, **11**, 2004–2010.
- J. Sun and S. Liu, *J. Food Biochem.*, 2015, **39**, 11–18.

- 14 P. Pongpamorn, C. Kiattisewee, N. Kittipanukul, J. Jaroensuk, D. Trisrivirat, S. Maenpuen and P. Chaiyen, *Angew. Chem., Int. Ed.*, 2021, **133**, 5813–5817. In this recent report, use of a carboxylic acid reductase (CAR) catalyzes esterification in an aqueous alcoholic medium. Both Mg^{2+} and ATP are required, and is imidazole, to generate the esters shown in modest yields.
- 15 (a) C. An, M. H. Shaw, A. Tharp, D. Verma, H. Li, H. Wang and X. Wang, *Org. Lett.*, 2020, **22**, 8320–8325; (b) S. Bojarra, D. Reichert, M. Grote, A. G. Baraibar, A. Dennig, B. Nidetzky, C. Mügge and R. Kourist, *ChemCatChem*, 2018, **10**, 1192–1201.
- 16 B. H. Lipshutz, S. Ghorai, A. R. Abela, R. Moser, T. Nishikata, C. Duplais, A. Krasovskiy, R. D. Gaston and R. C. Gadwood, *J. Org. Chem.*, 2011, **76**, 4379–4391. See also: D. Goswami, *Appl. Biochem. Biotechnol.*, 2020, **191**, 744–762.
- 17 R. C. Rodrigues and R. Fernandez-Lafuente, *J. Mol. Catal. B: Enzym.*, 2010, **64**, 1–22.
- 18 (a) M. Cortes-Clerget, N. Akporji, J. Zhou, F. Gao, P. Guo, M. Parmentier, F. Gallou, J. Y. Berthon and B. H. Lipshutz, *Nat. Commun.*, 2019, **10**, 2169; (b) N. Akporji, V. Singhanian, J. Dussart-Gautheret, F. Gallou and B. H. Lipshutz, *Chem. Commun.*, 2021, **57**, 11847–11850.
- 19 P. Villo, O. Dalla-Santa, Z. Szabó and H. Lundberg, *J. Org. Chem.*, 2020, **85**, 6959–6969.
- 20 The additive, $PhCF_3$, was not nearly as effective upon evaluation in the presence of other lipases used in this study (*Candida rugosa*, *Rhizopus niveus*, and *Burkholderia cepacia*).
- 21 (a) M. A. Cuendet, H. Weinstein and M. V. LeVine, *J. Chem. Theory Comput.*, 2016, **12**, 5758–5767; (b) D. E. Koshland, G. Némethy and D. Filmer, *Biochem.*, 1966, **5**, 365–385; (c) M. Jacque, J. Wyman and J. Changeux, *J. Mol. Biol.*, 1965, **12**, 88–118.
- 22 A. G. Sandstrom, Y. Wikmark, K. Engstrom, J. Nyhlen and J. E. Backvall, *Proc. Natl. Acad. Sci. U. S. A.*, 2012, **109**, 78–83.
- 23 (a) F. Khan, D. Lan, R. Durrani, W. Huan, Z. Zhao and Y. Wang, *Front. Bioeng. Biotechnol.*, 2017, **5**, 16; (b) A. M. Brzozowski, U. Derewendat, Z. S. Derewendat, D. M. Lawson, J. P. Turkenburg, F. Bjorkling, B. Huge-Jensen, S. A. Patkar and L. Thim, *Nature*, 1991, **351**, 491–494; (c) A. Roussel, S. Amara, A. Nyssölä, E. Mateos-Diaz, S. Blangy, H. Kontkanen, A. Westerholm-Parvinen, F. Carrière and C. Cambillau, *J. Mol. Biol.*, 2014, **426**, 3757–3772.
- 24 Y. Hayashi, *J. Org. Chem.*, 2021, **86**, 1–23.
- 25 Y. Hayashi, *Chem. Sci.*, 2016, **7**, 866–880.
- 26 N. Akporji, R. R. Thakore, M. Cortes-Clerget, J. Andersen, E. Landstrom, D. H. Aue, F. Gallou and B. H. Lipshutz, *Chem. Sci.*, 2020, **11**, 5205–5212.
- 27 B. S. Takale, R. R. Thakore, E. S. Gao, F. Gallou and B. H. Lipshutz, *Green Chem.*, 2020, **22**, 6055–6061.
- 28 A. B. Wood, M. Cortes-Clerget, J. R. A. Kincaid, B. Akkachairin, V. Singhanian, F. Gallou and B. H. Lipshutz, *Angew. Chem., Int. Ed.*, 2020, **132**, 17740–17746.
- 29 R. Chinchilla and C. Najera, *Chem. Soc. Rev.*, 2011, **40**, 5084–5121.
- 30 National Research Council (US) Chemical Sciences Roundtable, The Role of the Chemical Sciences in Finding Alternatives to Critical Resources: A Workshop Summary, *Replacing Critical Materials with Abundant Materials*, National Academies Press (US), Washington (DC), 2012, vol. 4.
- 31 We have no experimental proof at this time as to why enzymatic denaturation has yet to be seen independent of the amounts and nature of the metals present in the medium. Nonetheless, we suggest that the explanation may be attributed to the likelihood that the catalysts are predominantly localized in and around the micellar array; hence, as far as the enzyme is concerned, it may not be exposed to the high levels of potentially denaturing metals present, which is a textbook phenomenon.
- 32 P. T. Anastas and J. C. Warner, *Green Chemistry: Theory and Practice*, Oxford University Press, 1998.
- 33 R. A. Sheldon, D. Brady and M. L. Bode, *Chem. Sci.*, 2020, **11**, 2587–2605.