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

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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.

### 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). 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...
Table 1  Optimization of esterification in aqueous buffer + surfactant

| Entry | Conc. [M] | Alcohol (equiv.) | Temp. (°C) | Conversiona (%) |
|-------|-----------|-----------------|------------|----------------|
| 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               | 22b        | 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 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 (withstanding 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
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.

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

| Entry | Solvent<sup>a</sup> | Additive<sup>b</sup> (equiv.) | Yield<sup>c</sup> (%) |
|-------|---------------------|-----------------------------|----------------------|
| 1     | 2 wt% TPGS-750-M/0.01 M buffer | —                          | 0                    |
| 2     | 2 wt% TPGS-750-M/0.01 M buffer | PhCF₃ (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 | PhCF₃ (2)                  | 57                   |
| 5     | 0.01 M buffer (no surfactant) | PhCF₃ (1)                  | 0                    |

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

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**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).²⁸ This phenomenon was also observed in the lipase-catalyzed 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
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 PhCF₃ (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 sp² carbon (phenol, 35) or a sp³ 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 PhCF₃ (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”), and processing (“pot economy”). Importantly, while maintaining global concentrations that typically range between 0.25 and 1 M, 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 coupling 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/C led to intermediate acid 20. Subsequent lipase-catalyzed esterification with alcohol 45 gave gem-dibromocyclopropane 46, which was then reduced by nickel nanoparticles 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 performed specifically as a test of enzymatic compatibility with such high, indeed unsustainable amounts of both Pd and Cu. Thus, and
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.

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