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Exploitation of a *Candida antarctica* lipase B-catalysed *in situ* carboxylic acid activation method for the synthesis of acetanilides

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ABSTRACT

An efficient biocatalytic method has been developed which provides acetanilides in good yields which are otherwise inaccessible using *Candida antarctica* lipase B. The process exploits the enzyme-catalysed synthesis of an acyl donor and its *in situ* reaction with anilines. The method is potentially useful for the synthesis of bulky acetanilides since amide formation occurs through an active site-independent step.

1. Introduction

It has been mooted, by world-leading pharmaceutical companies that the development of a general, catalytic, environmentally friendly and scalable synthesis of (bulky) amides remains elusive [1]. Despite this, *N*-acylation reactions are used frequently in the preparation of drug candidates and some inevitably require preparation on a large scale. Due to the importance of amides, numerous synthetic methods exist in the chemist's tool-box to prepare them [2,3], but they typically involve stoichiometric activating agents and other coupling reagents, which require expensive removal and disposal at the end of the reaction, thus increasing their Environmental factor (E factor) and decreasing their atom efficiency [4], or they involve toxic and corrosive acid chlorides for their preparation [3]. Therefore, developing a method which uses fewer or catalytic reagents and obviates the need to isolate intermediates would be very desirable to the synthetic community. Biocatalysis is an ideal green and sustainable technique to answer this need and many research groups have attempted to achieve this goal. For example, Gotor-Fernandez et al. have shown CAL-B to be an ideal biocatalyst for the preparation of nitrogenated organic compounds [5], whilst van Pelt et al. have demonstrated that the aminolysis of various bulky methyl esters with amines occurs well with *Pseudomonas stutzeri* lipase [6]. However in these cases, as with other enzymes, limitations in the size of the enzyme's active site means that the size of the accepted substrates is also somewhat limited. The limitation due to the active-site's size can be partially overcome through the directed evolution of the enzyme [7,8], or, more simply, if the aminolysis reaction takes place outside of the active site itself. That is, if the enzyme can be used to catalyse the synthesis of an activated acyl donor (reminiscent of the reactivity of an acid chloride) then the amide forming step could take place outside of the active site with substrates which are too bulky to fit into it. Moreover, in this scenario, amines which cannot be catalysed directly by the enzyme, may well react with the activated acyl donor outside of the active site.

Oxime esters are known to react with amines in a reaction for the generation of amides [9–11], and the oxime ester can be prepared efficiently by enzymes from the corresponding oxime and an acyl donor [10,12–15]. Furthermore, Chen et al. have shown that unnatural amino acids can be incorporated into amino acid chains through the one-pot, enzyme-catalysed, reaction of an ester with benzaldehyde oxime and its further non-catalysed reaction *in situ* with an amine [15].

In order to develop a new method for the preparation of amides which require minimal processing and reduce waste production, we set out to expand upon this established work towards the synthesis of amides which cannot be prepared efficiently through the direct enzyme-catalysed reaction of an acid derivative and an amine.

The proposed catalytic cycle can be seen in Scheme 1. The enzyme (CAL-B) catalyses the reaction between the acyl donor (**1**) and acetone oxime (**3**) resulting in catalytic quantities of the reactive acetoneoxime ester (**2**). Once formed, the acetoneoxime ester

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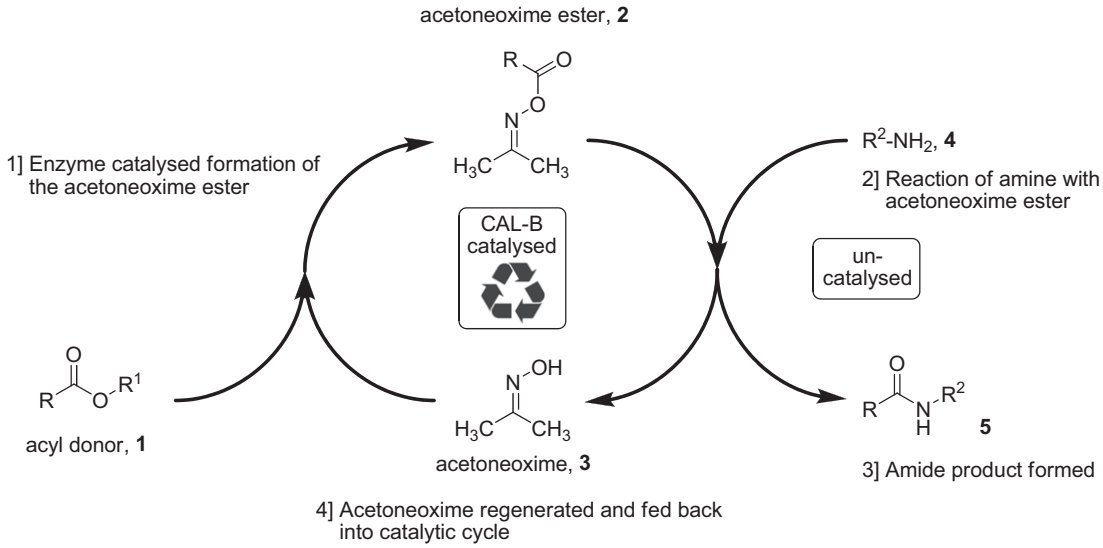


Scheme 1. Proposed catalytic cycle.

reacts with the amine resulting in the desired amide product (**5**), but importantly, releasing the oxime (**3**) to be fed back into the catalytic cycle again. An important feature of this process would be that the whole amide synthesis would be catalytic, requiring sub-stoichiometric quantities of both the enzyme and the acetone oxime. With equimolar quantities of only the acyl donor and amine being used, waste would be minimal, and the environmental impact would be further minimised if the enzyme could be reused in subsequent reactions.

2. Materials and methods

2.1. General synthetic procedures

Commercially available reagents were used as received without purification. Analytical thin layer chromatography (TLC) was performed with plastic-backed TLC plates coated with silica G/UV_{254}, in a variety of solvents. The plates were visualised by UV light (254 nm), *p*-anisaldehyde solution or $KMnO_4$ solution. Flash column chromatography was conducted with Davisil silica 60 Å (40–63 μm) under bellows pressure. Low-resolution mass spectra were recorded on a Thermo Finnigan LCQ Advantage MAX using electron spray ionisation (ESI). ^{1}H and ^{13}C NMR spectra were recorded on a BrukerAvance DPX 250 (250 MHz) a Bruker 300 (300 MHz) or a Bruker 400 (400 MHz) spectrometer. All chemical shifts (δ) are quoted in parts per million (ppm) relative to a calibration reference of the residual protic solvent; C*H*Cl_3 (δ_H 7.26, s) or DMSO-d_6 (δ_H 2.54, s) was used as the internal standard in ^{1}H NMR spectra, and ^{13}C NMR shifts were referenced using $CDCl_3$ (δ_C 77.0, t) or DMSO-d_6 (δ_H 39.5, m) with broad band decoupling and the *J* values are measured in Hertz.

All acetanilides are commercially available from either Sigma–Aldrich or Acros as depicted below.

2.2. Synthesis

2.2.1. Procedure for the chemical synthesis of the activated ester using acetyl chloride

To a solution of acetone oxime (2.55 g, 35 mmol, 1 equiv.) in dichloromethane (50 mL) was added pyridine (2.83 mL, 35 mmol, 1eq.) and acetyl chloride (2.48 mL, 35 mmol, 1 equiv.). The resulting solution was stirred in an ice bath for 6 h. After which, HCl (1 M, 50 mL) was added and the layers separated. The organic layer was washed with water (50 mL) and brine (50 mL), dried ($MgSO_4$) and the solvent evaporated to yield the activated ester as a colourless oil (1.82 g, 45%). R_f (50% EtOAc/Petrol): 0.53; ^{1}H NMR ($CDCl_3$, 250 MHz): δ 1.97 (s, 3H), 2.02 (s, 3H), 2.13 (s, 3H); ^{13}C NMR ($CDCl_3$, 62.5 MHz): δ 16.83, 19.49, 21.84, 163.61, 168.58; IR (neat): ν_{max}/cm^{-1} 876, 1200, 1368, 1754.

2.2.2. Procedure for the synthesis of the activated acetone oxime ester (*2*, *R*=CH_3) using CAL-B

To a solution of vinyl acetate (0.73 mL, 7.92 mmol, 2 equiv.) in methyl-*tert*-butyl ether (MTBE) (30 mL) was added acetone oxime (0.29 g, 3.96 mmol, 1 equiv.), CAL-B (3.0 g) and 4 Å molecular sieves (2.0 g). The resulting suspension was stirred at room temperature for 4 h. After which, it was filtered and the solvent was evaporated. The activated ester was purified by flash chromatography (SiO_2; 20% ethyl acetate in petroleum ether + 1% triethylamine) to yield the pure activated ester as a colourless oil (0.34 g, 76%). ^{1}H NMR was identical to that prepared chemically above.

2.2.3. Control 1 – general procedure for the reaction of the activated ester with amines

To a solution of the activated ester (1 equiv.) in MTBE (10 mL, 0.2 M) was added the amine (1 equiv., 0.2 M) and 4 Å molecular sieves (1.0 g per 1.73 mmol of activated ester). The resulting solution was stirred at room temperature for 4 h. After which, it was filtered and evaporated. The crude *N*-acylated product was purified by flash chromatography (SiO_2) to yield the pure amide.

2.2.4. Control 2 – general procedure for the CAL-B catalysed amide formation

To a solution of vinyl acetate (2 equiv.) in MTBE (10 mL) was added the amine (1 equiv., 0.2 M), CAL-B (1.5 g per 2.0 mmol of amine) and 4 Å molecular sieves (1.0 g per 2.0 mmol of amine). The resulting suspension was stirred at room temperature for 4 h. After which, it was filtered and evaporated. The crude *N*-acylated product was purified by flash chromatography (SiO_2) to yield the pure amide where applicable.

2.2.5. Control 3 – general procedure for the acetone oxime enhanced synthesis of amides

To a solution of vinyl acetate (2 equiv.) in MTBE (15 mL) was added acetone oxime (1 equiv.), CAL-B (1.5 g per 2.0 mmol of amine), the amine (1 equiv., 0.13 M) and 4 Å molecular sieves (1.0 g

per 2.0 mmol of amine). The resulting suspension was stirred at room temperature for 4 h. After which, it was filtered and evaporated. The crude *N*-acylated product was purified by flash chromatography (SiO_2) to yield the pure amide.

2.2.6. General procedure for the acetone oxime enhanced synthesis of amides with catalytic acetone oxime

To a solution of vinyl acetate (2 equiv.) in MTBE (15 mL) was added the amine (1 equiv., 0.13 M), CAL-B (1.5 g per 2.0 mmol of amine), acetone oxime (0.1 equiv.) and 4 Å molecular sieves (1.0 g per 2.0 mmol of amine). The resulting solution was stirred at room temperature for 4 h. After which, it was filtered and evaporated. The crude reaction product was purified by flash chromatography (SiO_2) to yield the pure amide.

2.3. Characterisation data

***N*-benzylacetamide (Sigma–Aldrich)**: off white crystals (62% yield); R_f (50% EtOAc/Petrol): 0.21; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.03 (s, 3H), 4.43 (d, 2H, J = 6.0 Hz), 7.28–7.34 (m, 5H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 23.07, 43.57, 127.38, 127.74, 128.61, 138.34, 170.35; MS (ESI$^+$, m/z): 149 (82%), 106 (99), 91 (30); IR (neat): ν_{max}/cm^{-1} 1546 (C=O stretching), 1646 (NH bending), 2926, 3289 (aromatic C—H).

Acetanilide (Sigma–Aldrich): white crystals (84% yield, eluted at 50% EtOAc/Petrol); R_f (50% EtOAc/Petrol): 0.27; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.18 (s, 3H), 7.10 (t, 1H, J = 7.5 Hz), 7.32 (t, 2H, J = 7.5 Hz), 7.50 (d, 2H, J = 6.0 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 24.5, 120.2, 124.3, 129.0, 138.1, 169.2. MS (ESI$^+$, m/z): 135 (30%), 136 (3), 93 (100); IR (neat): ν_{max}/cm^{-1} 1541 (C=O stretching), 1698 (NH bending), 3136, 3546 (aromatic C—H), 3614 (NH stretching).

4′-Hydroxyacetanilide (Sigma–Aldrich): off white crystals (48% yield, eluted at 60% EtOAc/Petrol); R_f (50% EtOAc/Petrol): 0.20; ^{1}H NMR (DMSO, 300 MHz): δ 2.01 (s, 3H), 6.70 (d, 2H, J = 9.0 Hz), 7.34 (d, 2H, J = 9.0 Hz); ^{13}C NMR (DMSO, 75 MHz): δ 23.8, 115.5, 121.3, 131.4, 153.7, 168.3; MS (ESI$^+$, m/z): 151 (33%), 109 (100); IR (neat): ν_{max}/cm^{-1} 1640 (NH bending), 3100 (Aromatic C—H).

4′-Methoxyacetanilide (Acros): off white crystals (69% yield, eluted at 40% EtOAc/Petrol); R_f (50% EtOAc/Petrol): 0.15; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.16 (s, 3H), 3.79 (s, 3H), 6.85 (d, 2H, J = 9.0 Hz), 7.38 (d, 2H, J = 9.0 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 24.4, 55.6, 114.0, 122.1, 131.1, 156.5, 168.7; MS (ESI$^+$, m/z): 164.91 (34%), 122.89 (65), 107.82 (99); IR (neat): ν_{max}/cm^{-1} 1509 (C=O stretching), 1556, 1604, 1644 (NH bending).

3′-Bromoacetanilide (Sigma–Aldrich): off white crystals (45% yield, eluted at 25% EtOAc/Petrol); R_f (50% EtOAc/Petrol): 0.28; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.18 (s, 3H), 7.15–7.22 (m, 3H), 7.4 (d, 1H, J = 6.0 Hz), 7.76 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 24.7, 118.5, 122.7, 122.9, 127.4, 130.4, 139.3, 168.7; MS (ESI$^+$, m/z): 213 (24%), 215 (23), 171 (99), 173 (93), 92 (56); IR (neat): ν_{max}/cm^{-1} 1538 (C=O stretching), 1662 (NH bending), 3292 (aromatic C—H).

4′-Bromoacetanilide (Acros): brown crystals (48% yield, eluted at 20% EtOAc/Petrol); R_f (50% EtOAc/Petrol): 0.24; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.17 (s, 3H), 7.47 (s, 4H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 24.7, 117.1, 121.7, 132.1, 137.1, 168.5; MS (ESI$^+$, m/z): 213 (26%), 215 (25), 171 (99), 173 (92), 92 (40); IR (neat): ν_{max}/cm^{-1} 1530 (C=O stretching), 1666 (NH bending), 3400 (aromatic C—H).

***N*-(benzo[*d*][1,3]dioxol-5-yl)acetamide (Sigma–Aldrich)**: brown crystals (64% yield, eluted at 50% EtOAc/Petrol); R_f (50% EtOAc/Petrol): 0.26; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.14 (s, 3H), 5.94 (s, 2H), 6.71–6.78 (m, 2H), 7.20 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 24.4, 101.3, 103.1, 108.3, 113.4, 132.4, 144.3, 148.0, 168.9; MS (ESI$^+$, m/z): 179 (48%), 137 (99), 136 (30); IR (neat): ν_{max}/cm^{-1} 1543 (C=O stretching), 1635 (NH bending), 3307 (aromatic C—H).

4′-Methylacetanilide (Sigma–Aldrich): brown crystals (90% yield, eluted at 40% EtOAc/Petrol); R_f (50% EtOAc/Petrol): 0.33; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.16 (s, 3H), 2.30 (s, 3H), 7.10 (d, 2H, J = 9.0 Hz), 7.37 (d, 2H, J = 9.0 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 20.9, 24.55, 120.2, 129.5, 134.0, 135.5, 168.7; MS (ESI$^+$, m/z): 149 (50%), 106 (98), 107 (100); IR (neat): ν_{max}/cm^{-1} 1660 (C=O stretching), 1548 (NH bending), 3289 (NH stretching).

4′-*tert*-Butylacetanilide (Sigma–Aldrich): brown crystals (41% yield, eluted at 35% EtOAc/Petrol); R_f (50% EtOAc/Petrol): 0.28; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.30 (s, 9H), 2.17 (s, 3H), 7.32–7.42 (m, 4H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 24.5, 31.4, 34.3, 120.0, 125.8, 135.4, 147.3, 169.0; MS (ESI$^+$, m/z): 191 (26%), 176 (66), 134 (100); IR (neat): ν_{max}/cm^{-1} 1515 (C=O stretching), 1664 (NH bending), 3251 (aromatic C—H), 1375 (*tert*-butyl).

4′-Butoxyacetanilide (Sigma–Aldrich): white crystals (41% yield, eluted at 30% EtOAc/Petrol); R_f (50% EtOAc/Petrol): 0.20; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.96 (t, 3H, J = 7.5 Hz), 1.44–1.54 (m, 2H), 1.70–1.79 (m, 2H), 2.15 (s, 3H), 3.93 (t, 2H, J = 6.0 Hz), 6.84 (d, 2H, J = 9.0 Hz), 7.08 (s, 1H), 7.36 (d, 2H, J = 9.0 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 13.9, 19.3, 24.2, 31.4, 68.0, 114.7, 122.1, 131.0, 156.1, 168.7; MS (ESI$^+$, m/z): 207 (24%), 151 (16), 109 (100); IR (neat): ν_{max}/cm^{-1} 1541 (C=O stretching), 1658 (NH bending), 3305 (aromatic C—H), 1368 (alkyl chain).

***N*-(2,3-dihydro-1H-inden-5-yl)acetamide (Acros)**: light yellow crystals (65% yield, eluted at 30% EtOAc/Petrol); R_f (50% EtOAc/Petrol): 0.32; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.01–2.11 (m, 2H), 2.16 (s, 3H), 2.83–2.91 (q, 4H, J = 9.0 Hz), 7.14 (s, 2H), 7.44 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 23.9, 25.7, 32.2, 32.8, 116.8, 118.5, 124.1, 136.3, 139.9, 144.8, 169.3; MS (ESI$^+$, m/z): 175 (46%), 132 (100), 133 (84); IR (neat): ν_{max}/cm^{-1} 1538 (C=O stretching), 1656 (NH bending), 3276 (aromatic C—H).

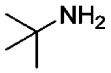
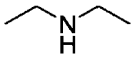
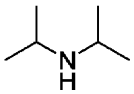
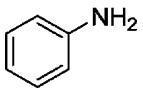
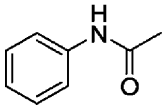
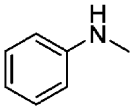
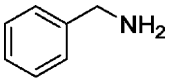
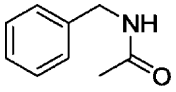
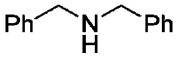
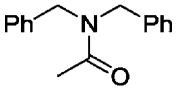
2-Acetamido-5,6,7,8-tetrahydronaphthalene (Sigma–Aldrich): light brown crystals (54% yield, eluted at 25% EtOAc/Petrol); R_f (50% EtOAc/Petrol): 0.31; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.79 (s, 4H), 2.16 (s, 3H), 2.73 (s, 4H), 7.00 (d, 1H, J = 9.0 Hz), 7.19 (d, 1H, J = 9.0 Hz), 7.29 (s, 1H, J = 7.3); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 23.1, 23.3, 24.3, 28.9, 29.5, 117.9, 120.9, 129.3, 133.2, 135.4, 137.6, 169.1; MS (ESI$^+$, m/z): 189 (60%), 147 (100), 119 (83); IR (neat): ν_{max}/cm^{-1} 1654 (C=O stretching), 1537 (NH bending), 3244 (NH stretching).

3. Results and discussion

Initial investigation focussed on the use of methyl esters as the acyl donor, and included both methyl benzoate and methyl phenylacetate, however, in our hands, the reversibility of the reaction was problematic wherein the methanol liberated in the CAL-B catalysed step was more reactive than the amines being studied and thus it reacted with the acetoneoxime ester (**2**) giving starting ester (**1**) back again. Despite the use of 4 Å molecular sieves to remove the methanol as it formed [6], the reaction could not be optimised with methyl esters using our chosen conditions. As such, we resorted to using vinyl acetate as an irreversible acyl donor in order to acquire proof of principle of the proposed catalytic cycle shown in Scheme 1. An initial screen of the amines studied can be seen in Table 1.

All reactions were compared to controls such that control 1 involves the direct reaction of the amine (**4**) with chemically synthesised acetoneoxime ester (**2**) to determine if amide formation is possible and worthy of further study; control 2 involves the reaction between vinyl acetate (**1**, R=CH_3, R^1=CH=CH_2) and the amine (**4**) catalysed by CAL-B to determine the level of the background reaction and thus gain a benchmark to observe improvements made with the acetoneoxime catalysed reaction; and control 3 involves a one-pot reaction with all components necessary for the proposed catalytic cycle. In the initial reactions equimolar quantities of acetone oxime and the amine were used before being reduced to catalytic amounts (0.1 equiv., **3**).

Table 1
Initial screen of amines studied.

Entry	Amine	Product	Control 1[a] Yield (%)[d]	Control 2[b] Yield (%)[d]	Control 3[c] Yield (%)[d]	Increase (control 3/control 2)
1		–	No reaction	n.d.	n.d.	–
2		–	No reaction	n.d.	n.d.	–
3		–	No reaction	n.d.	n.d.	–
4			52	13	84	6.46
5		–	No reaction	n.d.	n.d.	–
6			50	42	62	1.48
7			Trace product	6	n.d.	–

[a] Control 1: **2** (1 equiv.), **4** (1 equiv.), 4 Å molecular sieves (1 g) in MTBE (10 mL), 4 h.
[b] Control 2: vinyl acetate (2 equiv.), **4** (1 equiv.), 4 Å molecular sieves (1 g), CAL-B (1.5 g) in MTBE (10 mL), 4 h.
[c] Control 3: vinyl acetate (2 equiv.), **4** (1 equiv.), 4 Å molecular sieves (1 g), **3** (1 equiv.), CAL-B (1.5 g) in MTBE (15 mL), 4 h.
[d] Isolated yield of pure material. n.d.: not determined.

As can be seen in Table 1, the majority of amines (entries 1, 2, 3, 5 and 7) did not react with the activated ester (**2**) at all (control 1), as such these amines were not studied further, the rationale being that if they were unable to react with **2** in a control reaction, they would not react with it *in situ* either. In case of benzylamine, the one-pot yield was only slightly better (entry 6, Table 1, 62%), compared to the CAL-B catalysed amidation (control 2, 42%). However, the presence of two benzyl groups (entry 7, Table 1) completely prevented amide formation (control 1). The result of the enzyme catalysed amidation in this case also gave a very low yield (control 2, 6%) possibly indicating a limitation to our method and the limited active site area of CAL-B being unable to accommodate this bulky amine. Nevertheless, success was observed for aniline and benzylamine (entries 4 and 6, respectively). For example, when the yield of the aniline reaction catalysed by CAL-B alone (entry 4, control 2, Table 1) was compared to the yield obtained when acetone oxime (1 equiv.) was added to the reaction (control 3) a 6.46-fold increase was observed (13% to 84%), thus highlighting the potential for this reaction as a method to prepare inaccessible CAL-B-catalysed products. Since it is reported that anilines are not suitable for CAL-B catalysed amidations [16,17] and do not readily react with vinyl acetate [18,19], along with the known potential of acetanilides as chemical hybridising agents [20], and as antiparasitic agents [21] we thought that this reaction was worthy of further study. Moreover, the stark difference observed between the results depicted in entries 4 and 5 (Table 1) reveal that there appears to be selectivity for primary over secondary anilines. Presumably, the increased steric hindrance of the secondary amine overrides the increase in nucleophilicity gained by the extra alkyl group, a result which could potentially add further value to this method.

With these preliminary results in hand, we set out to explore the range of anilines that would participate in this reaction, but importantly, we wanted to analyse the difference between the CAL-B catalysed reaction with (control 3) and without (control 2) acetone oxime (both in stoichiometric and in catalytic quantities) to determine if an increase in yield could be obtained with the added oxime. Table 2 outlines these results.

All reactions carried out were compared to the CAL-B catalysed reaction, without acetone oxime being added (control 2, Table 2), as well as a background control between the amines and vinyl acetate, in the absence of CAL-B and acetone oxime, the results of which gave no amide products at all (data not shown) [18]; all controls were carried out in order to prove the efficacy of enzyme-catalysed ester activation method. In this way, products which are not obtainable with CAL-B alone can be identified and obtained simply by the addition of acetone oxime.

The results, although no attempt at optimisation was made, do indicate an increase in the yield of the acetanilides when the reaction is performed in the presence of acetone oxime to activate the vinyl acetate, Table 2 (control 3/control 2).

Aniline (entry 1, Table 2) produces the amide in 84% yield with acetone oxime (1 equiv.) compared to the CAL-B catalysed amidation (control 2, 13%). To date, there are very few reports on the use of CAL-B to prepare *N*-phenylacetamide from aniline [16,17]; a result confirmed by us wherein the CAL-B catalysed amidation gave only 13% yield of *N*-phenylacetamide (Table 2, entry 1). Disappointingly, the yield of amide formed with catalytic acetone oxime (entry 1, Table 2) was only 35%, however, run over the same time (4 h) as the controls, this yield does prove the concept outlined in Scheme 1.

To further prove the importance of acetone oxime in these reactions, an experiment with vinyl acetate (1 equiv.), aniline (1 equiv.) and CAL-B was carried out in MTBE, without the addition of acetone oxime. After 4 h there was negligible amide formation, confirmed

Table 2
Studies with aniline derivatives.

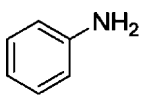
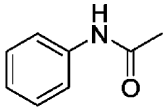
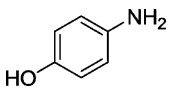
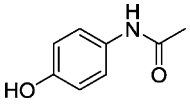
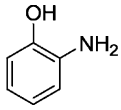
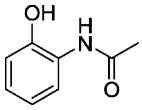
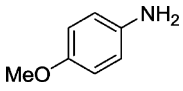
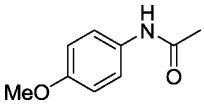
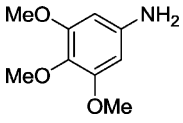
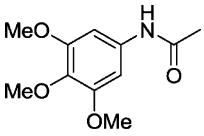
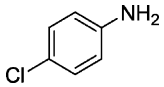
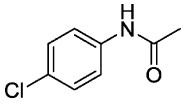
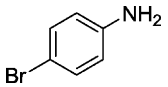
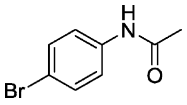
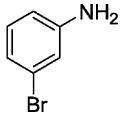
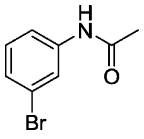
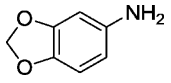
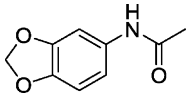
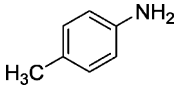
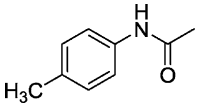
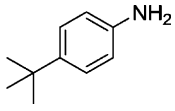
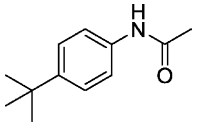
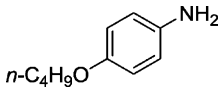
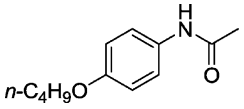
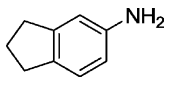
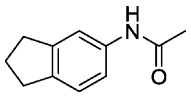
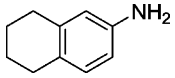
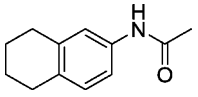
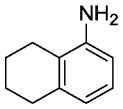
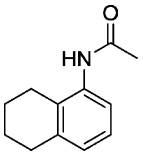
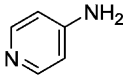
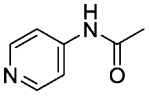
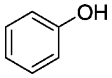
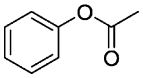
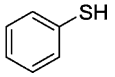
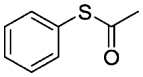
Entry	Amine	Product	Control 1[a] Yield (%)[d]	Control 2[b] Yield (%)[d]	Control 3[c] Yield (%)[d]	Increase (control 3/control 2)	Catalytic acetone oxime[e] Yield (%)[d]
1			52	13	84	6.46	35
2			54[f]	<1%	72	>72	No reaction
3			Trace product	n.d.	n.d.	–	n.d.
4			v75	23	69	3.00	72
5			No reaction	n.d.	n.d.	–	n.d.
6			Trace product	n.d.	n.d.	–	n.d.
7			46	7	48	6.86	33
8			11	7	45	6.43	24
9			40	43	64	1.49	40
10			88	55	90	1.64	52
11			88	11	41	3.73	27
12			86	7	41	5.86	29
13			83	18	65	3.61	35
14			84	35	54	1.54	41

Table 2 (*Continued*)

Entry	Amine	Product	Control 1[a] Yield (%)[d]	Control 2[b] Yield (%)[d]	Control 3[c] Yield (%)[d]	Increase (control 3/control 2)	Catalytic acetone oxime[e] Yield (%)[d]
15			No reaction	n.d.	n.d.	–	n.d.
16			No reaction	n.d.	n.d.	–	n.d.
17			No reaction	n.d.	n.d.	–	n.d.
18			No reaction	n.d.	n.d.	–	n.d.

[a] Control 1: **2** (1 equiv.), **4** (1 equiv.), 4 Å molecular sieves (1 g) in MTBE (10 mL), 4 h.
[b] Control 2: vinyl acetate (2 equiv.), **4** (1 equiv.), 4 Å molecular sieves (1 g), CAL-B (1.5 g) in MTBE (10 mL), 4 h.
[c] Control 3: vinyl acetate (2 equiv.), **4** (1 equiv.), 4 Å molecular sieves (1 g), **3** (1 equiv.), CAL-B (1.5 g) in MTBE (15 mL), 4 h.
[d] Isolated yield of pure material.
[e] Catalytic acetone oxime: as control 3 but with **3** (0.1 equiv. instead of 1 equiv.).
[f] Yield based on crude NMR. n.d.: not determined.

by NMR of the reaction mixture. After NMR analysis, one equivalent of acetone oxime was added to the same reaction mixture and the reaction was continued for a further 4 h. After which the NMR was taken again which showed the formation of acetanilide had occurred along with the activated ester (**2**, R=CH_3); an experiment which demonstrates the necessity of acetone oxime in this protocol.

The presence of the hydroxyl group at position-4 of aniline (entry 2) also reacted well in this protocol with stoichiometric acetone oxime (control 3, 48%) compared to the CAL-B catalysed amidation (control 2) which gave negligible yield of the amide product. No *O*-acetylation was observed, but lipase catalysed synthesis of phenol esters are rare [17]. Whilst it was presumed that the electron donating hydroxyl group would increase the nucleophilicity of the amine, its yield was less than the corresponding reaction with aniline, suggesting that factors other than resonance affect the reactivity of this amine. Strangely, no reaction occurred at all when catalytic acetone oxime was used, a result which requires understanding if the method is to be exploited further. In contrast, when the hydroxyl group is in the 2-position (entry 3), negligible yield of the amide product was obtained even in its reaction with the activated ester (control 1). Presumably such lack of reaction was either due to steric hindrance of the hydroxyl group preventing its reaction with the activated ester, or inter- and intramolecular hydrogen bonding taking place, preventing reaction; as a result of this lack of reactivity in control 1, further reactions of control 2 and 3 were not carried out with this amine.

Gratifyingly, the presence of a methoxy or butoxy group at position-4 on the ring (entries 4 and 12, respectively) also reacted well in the one-pot synthesis (control 3, 69% and 41%, respectively) compared to the CAL-B catalysed amidation (control 2, 23% and 7%, respectively), suggesting that further functionalisation is tolerated at this position. Somewhat pleasingly, with catalytic acetone oxime, the yield of the amide products were 72% and 29%, respectively.

Increasing the number of methoxy groups from one (entry 4) to three (entry 5) was also detrimental, such that, 3,4,5-trimethoxyaniline (entry 5) did not give the amide product at all upon reaction with the activated ester (control 1) and was therefore not studied further.

The inclusion of halogen atoms also seems to have an effect on yield. For example, the reaction of 4-chloroaniline (entry 6) with the activated ester (control 1) gave negligible yield, indicating the dominance of an electron withdrawing inductive effect, compared to electron donating resonance effect, at making the amine less reactive than aniline.

However, the presence of the less electronegative bromine in 4-bromoaniline gave a better yield of the corresponding amide in the one-pot synthesis, that is, 48% (entry 7), compared to the CAL-B catalysed amidation (control 2, 7%) suggesting that the electronic effects are less dominating here, although the yield is also reduced compared to aniline (entry 1). Furthermore, the presence of bromine at position-3 (3-bromoaniline, entry 8), gives a similar yield (45%) compared to that obtained from 4-bromoaniline (48%). In a similar manner, these two derivatives also gave comparable yields with catalytic acetone oxime (33% for 4-bromoaniline and 24% for 3-bromoaniline).

In contrast to the results in entry 5 the presence of two oxygen atoms in 3,5-methylenedioxyaniline (entry 9) also gave an increased yield (64%) compared to the CAL-B catalysed amidation without acetone oxime (43%), and this reaction also gave the amide in 40% yield with catalytic acetone oxime; although in this case the result could simply be background reaction.

The presence of an activating methyl group (entry 10) gave good yields with stoichiometric acetone oxime (90%) compared to the lipase catalysed reaction (55%), whilst catalytic acetone oxime gave 52% of the acetanilide. Similarly, the presence of a *tert*-butyl group (entry 11) gave an increased yield of the amide (41%) over the lipase catalysed amidation (control 2, 11%) and also produced the amide in 27% yield with catalytic acetone oxime, results which highlight the potential of this method for preparing bulky amides.

Other more hindered amines, such as, 5-aminoindan (entry 13) and 5,6,7,8-tetrahydro-2-naphthylamine (entry 14) reacted well with the activated ester with stoichiometric (65% and 54%, respectively) and catalytic acetone oxime (35% and 41%, respectively), in comparison to the CAL-B only catalysed amidation (control 2, 18% and 35%, respectively); amides which are reported to be intermediates for the synthesis of tricyclic 1,2,4-triazine oxides as potential cancer treatments [22]. However, the isomeric amine (entry 15)

did not react with the activated ester, presumably due to the steric hindrance of the adjacent methylene group hampering nitrogen's attack on the electrophilic carbonyl of the activated ester [23].

Furthermore, the presence of nitrogen in the ring (entry 16) did not give rise to any amide product. This could be correlated to the delocalisation of N(sp^3) electrons into the ring causing a decrease in the electron density on this nitrogen atom and thus a decrease in its nucleophilicity. No N(sp^2)-acetylation was observed during this reaction; only activated ester was present in the crude NMR of the reaction, despite the similarity of this amine to 4-dimethylaminopyridine (DMAP) [24].

In addition to anilines we briefly looked at the reactions of phenol and thiophenol with the activated ester to see if this method would be suitable for the preparation of esters and thioesters. In the event, both phenol (entry 17) and thiophenol (entry 18) gave negligible yields of the acetylated product indicating their poorer nucleophilicity compared to their nitrogen-based aniline analogues and lack of utility in this protocol as it stands.

4. Conclusion

In summary, anilines which are known to be less reactive towards CAL-B catalysed amide formation were acetylated well with the developed methodology since the amide bond forming reaction seems to occur outside the active site of the enzyme. Since this reaction does not rely on the size of the enzymes active site it is possible that it could be developed into a useful biocatalytic method for preparing bulky amides. Optimisation and determination of the scope and limitations of this method are currently being pursued.

Acknowledgement

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.molcatb.2012.07.007.

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