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COMMUNICATION

# First Report of Bio-catalytic *N*-formylation of Amines Using Ethyl formate

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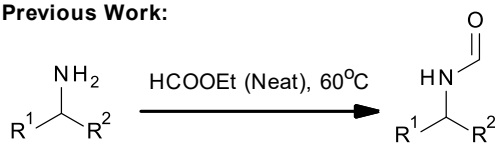
**A Bio-catalyzed *N*-formylation reaction of different amines has been developed using ethyl formate as formylating agent. This protocol provides a facile and convenient strategy featuring mild reaction condition, high efficacy, broad substrate scope and recyclability of lipase. This method also works on a large scale in high yield.**

*N*-formyl derivatives of amines are compound of considerable interest due to their wide application in the synthesis of important biologically active compounds.[1] They have also been employed as key reagents for the Vilsmeier formylation reaction,[2] amino acid protecting group in peptide synthesis,[3] and as Lewis base catalysts for allylation[4] or hydrosilylation[5] of carbonyl compounds. The most commonly used method for the preparation of these compounds relies on formylation of amines using a formylating agent such as chloral,[6] acetic formic anhydride,[7] formic acid,[8] ammonium formats,[9] cyanide,[10] formate esters,[11] *N,N*-dimethylformamide,[12] formyl fluoride,[13] aldehydes[14] and methanol.[15] Use of carbon monoxide and carbon dioxide is also reported in literature.[16] However, these methods suffer from several limitations such as harsh conditions, use of expensive metal- or organo-catalysts, tedious procedures or prolonged reaction time.[17] Therefore, the development of mild and environmentally friendly methods for *N*-formylation of amines remains highly desirable for organic transformations. Herein, we describe a simple and convenient method for *N*-formylation of amines using ethylformate catalyzed by lipase acrylic resin from *Candida antarctica*; Novozyme 435® CALB.

In the recent years, use of biocatalysts in organic synthesis has gained huge attention due to their efficacy and green nature.[18] Lipases are highly stable and readily available enzymes, which remains active even under unfavorable conditions. The most significant use of lipase in organic chemistry is kinetic resolution of racemic amines or alcohols.[19] In addition, they also catalyze a variety of common organic reactions such as esterification, inter-esterification, transesterification, alcoholysis, acidolysis, aminolysis and hydrolysis of organic carbonates.[20] Though lipase catalyzed *N*-acetylation of amines are known in the literature,[19] to the best of our knowledge there is no report for *N*-formylation catalyzed by lipase. Use of ethyl formate for *N*-formylation of amines is known but it requires higher temperature and longer reaction time (Scheme-1).[21] This prompted us to explore lipase catalyzed *N*-formylation of amines using ethyl formate under mild reaction conditions.

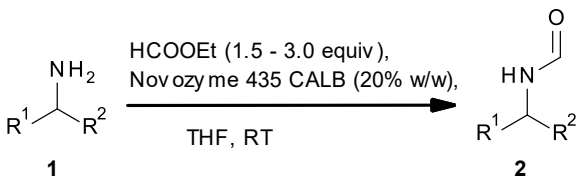
**Previous Work:**




R¹, R² = Alkyl, Aryl, Heteroaryl, Benzyl

**Scheme 1** *N*-formylation of amines with ethyl formate at 60°C.

**This Work:**




**1** **2**

R¹, R² = Alkyl, Aryl, Heteroaryl, Benzyl

**Scheme 2** *N*-formylation of amines with ethyl formate catalysed by lipase; Novozyme 435® CALB at RT.

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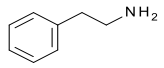
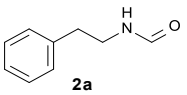
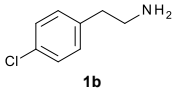
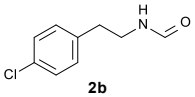
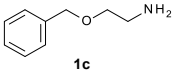
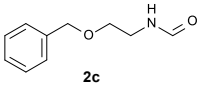
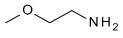
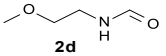
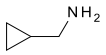
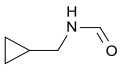
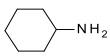
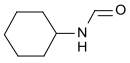
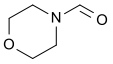
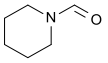
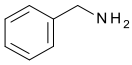
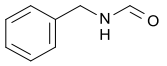
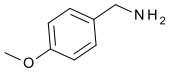
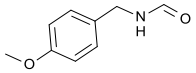
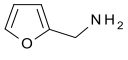
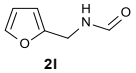
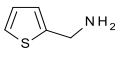
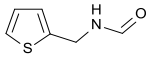
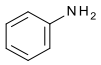
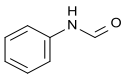
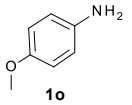
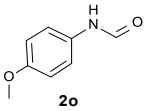
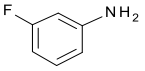
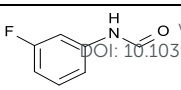
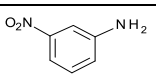
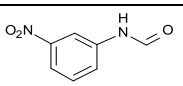
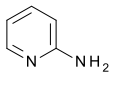
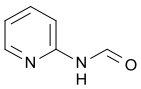
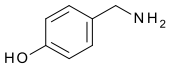
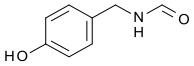
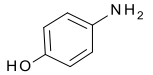
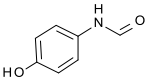
Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/x0xx00000x

**Table 1** Formylation of amines with ethyl formate catalysed by lipase[a]

| Entry | Amine | Product | Yield[c] |
|---|---|---|---|
| 1 |  **1a** |  **2a** | 99 |
| 2 |  **1b** |  **2b** | 99 |
| 3 |  **1c** |  **2c** | 99 |
| 4 |  **1d** |  **2d** | 95 |
| 5 |  **1e** |  **2e** | 94 |
| 6 |  **1f** |  **2f** | 98 |
| 7 |  **1g** |  **2g** | 94 |
| 8 |  **1h** |  **2h** | 91 |
| 9 |  **1i** |  **2i** | 90 |
| 10 |  **1j** |  **2j** | 99 (8[d]) |
| 11 |  **1k** |  **2k** | >99[e] |
| 12 |  **1l** |  **2l** | 99 |
| 13 |  **1m** |  **2m** | 99 |
| 14 |  **1n** |  **2n** | 87[b] |
| 15 |  **1o** |  **2o** | 97[b] |
| 16 |  **1p** |  **2p** | 80[b,f] |
| 17 |  **1q** |  **2q** | 29[b,f] |
| 18 |  **1r** |  **2r** | 78[b,f] |
| 19 |  **1s** |  **2s** | 91 |
| 20 |  **1t** |  **2t** | 90[b] |
| 21 |  **1u** |  **2u** | 80[b,g] |
| 22 |  **1v** |  **2v** | 94 |
| 23 |  **1w** |  **2w** | 95 |
| 24 |  **1x** |  **2x** | 92 |
| 25 |  **1y** |  **2y** | 95 |
| 26 |  **1z** |  **2z** | 90 |

[a]Reaction condition (Method A): amine (5 mmol), ethyl formate (7.5 mmol), Novozyme® 435 CALB (20%w/w), THF, RT, 1-8 h.
[b]Reaction condition (Method B): amine (5 mmol), ethyl formate (neat), Novozyme® 435 CALB (20%w/w), RT, 1-8 h.
[c]Isolated yield.
[d]Method A in absence of lipase (HPLC conversion).
[e]Done on 60 mmol scale.
[f]Reaction time 24 h.
[g]1.0 equiv of ethyl formate used.

Initially, we attempted *N*-formylation of benzyl amine using excess ethyl formate and 50% w/w of lipase, similar to the condition known for *N*-acetylation catalyzed by lipase.[19] To our gratification, the reaction went to completion within 1 h to provide desired *N*-formyl benzyl amine in 99% of isolated yield. With this exciting result in hand, we tried other formylating

agents such as formic acid, *N,N*-dimethylformamide, ammonium formate and trimethyl orthoformate. However, none of these formylating agents provided the desired product in considerable yield except formic acid, which took more than 48 h for complete conversion. We next explored the impact of lipase stoichiometry on the outcome of the reaction. We observed that excess lipase was beneficial for faster conversion. Conversely, a lower amount of lipase led to substantially slower conversion. As a control, we also measured the background reaction in the absence of lipase confirming its vital role (Table 1, entry 10). To evaluate the effect of solvent, we screened solvents of different polarity, such as dichloromethane, 1,2-dichloroethane, 1,4-dioxane, *N,N*-dimethylformamide, tetrahydrofuran, water and cyclohexane. Tetrahydrofuran found to be the most suitable solvent for the reaction. Our subsequent aim was to reduce the amount of ethyl formate. The best result was obtained 1.5 equivalent of ethylformate and 20% w/w of lipase in THF at room temperature (Scheme 2).[22]

To demonstrate the versatility of the method a wide number of aliphatic, benzylic, aromatic and hetero-aromatic amines were subjected to the novel reaction conditions (Table 1). In general, the reaction proceeds efficiently with various amines to provide the corresponding *N*-formylated product in good to excellent yield within very short period of time. Aliphatic primary and secondary amines underwent smooth *N*-formylation and gave the product in 90-99% yields (Table 1, entry 1-9). We next investigated the scope of *N*-formylation of benzyl amines. The reaction with benzyl amines proceeded even more smoothly to afford the desired product in 99% yield in a shorter reaction time (Table 1, entry 10-13). We also studied the reaction on a large scale (60 mmol) synthesis of *N*-[(4-methoxyphenyl) methyl]formamide (**2k**), which afforded the *N*-formylated product in quantitative yield (Table 1, entry 11). Anilines were found to be low yielding under the optimized conditions. However, when aniline was treated with neat ethyl formate using 20% w/w lipase, gave the *N*-formyl product (**2n**) in 87% yield (Table 1, entry 14). Aniline with electron donating group provided excellent yield (Table 1, entry 15) whereas anilines having electron withdrawing groups such 3-fluoroaniline or 3-nitroaniline afforded product in moderate yield (Table 1, entry 16-17). In the same way, heteroarylamine 2-aminopyridine provided *N*-(2-pyridyl)formamide (**2r**) in 78% yield (Table 1, entry 18). Excellent chemo-selectivity of amino groups over aryl-hydroxyl groups was observed with only *N*-formylated product was obtained for both the molecules examined.[23] (Table 1, entry 19-20). Similarly, controlled chemo-selectivity was observed for aliphatic amines over aromatic amines in the reaction of 4-amino benzylamine (Table 1, entry 21) to afford the corresponding *N*-[(4-aminophenyl)methyl]formamide (**2u**) in 80% yield.

The methodology was further extended to optically pure chiral amines without epimerization at the stereo-genic center as determined by chiral HPLC (Table 1, entry 24-26). However, attempts[24] for kinetic resolution of racemic amines (Table 1, entry 22-23) under the same condition was not successful though it provided corresponding *N*-formylated products in very good yields without any change in the enantiomeric ratio. Preliminary steady-state kinetic parameters were measured for the Novozyme 435® CALB catalyzed *N*-formylation reaction of one of the representative substrates 4-methoxybenzylamine. The values of $K_m$ (Michaelis-Menten constant) and $K_{cat}$ of the lipase in the reaction were calculated from the Lineweaver-Burk plot (Fig. 1) and presented in Table 2 (for details of the kinetic experiments see supporting information).

**Table 2** Kinetic constants for *N*-formylation reaction of 4-methoxybenzylamine with ethyl formate using Novozyme 435® CALB.

| $V_{max}$ (mole $min^{-1}$) | $K_m$ (M) | $K_{cat}$ ($min^{-1}$) | $K_{cat}/K_m$ ($mole^{-1}$ $min^{-1}$) |
|---|---|---|---|
| 0.100 | 1.8 | $0.42X10^2$ | $0.23X10^2$ |

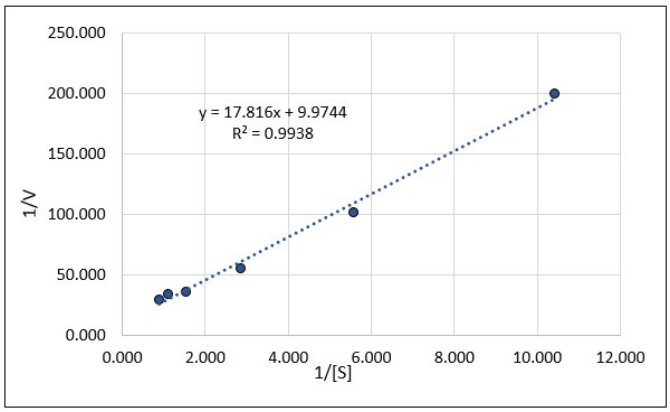



**Fig. 1** Lineweaver-Burk plot for *N*-formylation reaction of 4-methoxybenzylamine with ethyl formate using Novozyme 435® CALB

Next we studied the reusability of the lipase for the *N*-formylation reaction. After completion of the reaction the lipase was recovered by filtration, washed with tetrahydrofuran and dried under high vacuum at room temperature. Reusability of lipase was examined up to 10 consecutive cycles, and minimal loss of activity was observed. In all the 10 batches starting material was fully consumed to afford desired *N*-formylated product in quantitative yield, though longer reaction times were required (Fig. 2).

Noteworthy, most of the reaction masses were purified by simple filtration and evaporation of the volatiles to provide the desired product in very high purity making the methodology even more attractive.

In conclusion, we have successfully demonstrated the novel application of lipase for *N*-formylation of different amines using ethyl formate, which is mild, robust, high yielding and environmentally safe. The conditions were proven to be chemo-selective and applicable to chiral substrates without epimerization. The protocol is particularly advantageous for

ease, quick, and quantitative separation of the lipase for reuse. Preliminary kinetic parameters of one of the representative substrates have also been reported. Moreover, this method can be useful for the large scale synthesis of *N*-formyl amines. Efforts are currently underway in our research group to apply this method to the construction of other potentially valuable organic molecules.

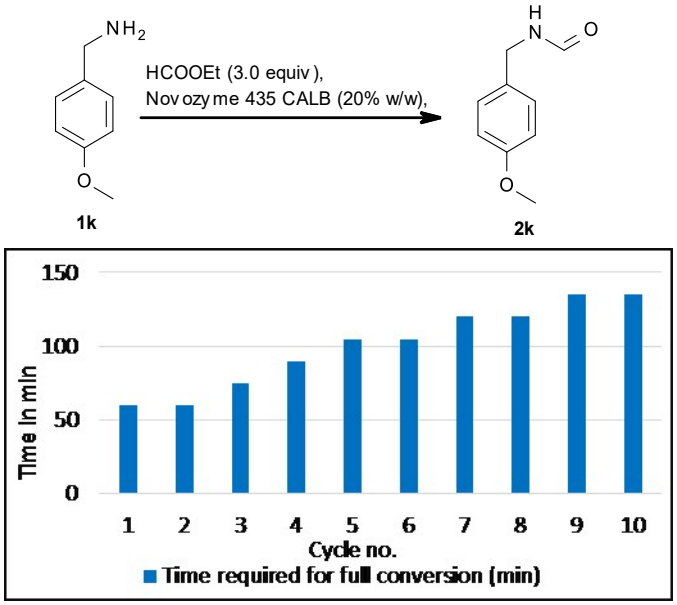



**Fig. 2** Recycling of the lipase for *N*-formylation of 4-methoxybenzylamine.

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22 General procedure for the *N*-formylation of amines:
**Method-A**: To a mixture of amine (5.0 mmol) and ethyl formate (7.5 mmol) in THF (5.0 ml) was added Novozyme 435® CALB (Lipase acrylic resin from *Candida antarctica* ≥5,000 U/g, recombinant, expressed in Aspergillus niger) (20% w/w), and the mixture was stirred at room temperature. After complete consumption of starting material (1- 8 h), the reaction mixture was filtered off, solid was washed with THF and the combined organic layers was evaporated to afford the *N*-formylated product in pure form.
**Method-B:** To a mixture of amine (5.0 mmol) and ethyl formate (15.0 to 25.0 mmol) was added Novozyme 435® CALB (20% w/w) and the reaction mixture was stirred at room temperature for 1-8 h or until starting material was fully consumed. The reaction mixture was diluted with THF and the product was isolated following the same procedure as **Method A**.

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24 For experimental details, see supporting information.