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**Note**

# Biocatalytic acylation of Efavirenz intermediate: Unexpected novel product formation

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During attempted biocatalytic resolution of (*R*/*S*)-5-chloro-α-(cyclopropylethynyl)-2-amino-α-(trifluoromethyl) benzenemethanol containing a tertiary hydroxyl group, a novel, unexpected benzoxazine derivative (Efavirenz analogue) is formed during the lipase B from *Candida antarctica* (Novozyme -435) catalyzed reaction of Efavirenz intermediate (*R*/*S*)-5-chloro-α-(cyclopropylethynyl)-2-amino-α-(trifluoromethyl) benzenemethanol **1** using vinyl acetate.

**Keywords:** Biocatalytic acylation, Efavirenz, benzoxazine derivative, lipase B

Effective treatment regimens for the human immunodeficiency virus (HIV-1) infection have included both HIV protease and reverse transcriptase inhibitors. Reverse transcriptase inhibitors are of two types: nucleoside reverse transcriptase inhibitors (NRTIs) such as AZT, 3TC and DDI, and non-nucleoside reverse transcriptase inhibitors (NNRTIs) such as nevirapine[1], delavirdine[2] and Efavirenz[3] (**Figure 1**). Efavirenz (Sustiva™), a potent reverse transcriptase inhibitor was discovered at Merck Research Laboratories[3]. Efavirenz was the first anti-HIV drug approved by the FDA (September 17, 1998) for once a day dosing when used in a combination regimen in both adult and pediatric patients[4].

Importance of Efavirenz led to the development of several strategies for its synthesis[5-7]. Most of the synthetic strategies involve the use of the intermediate (*S*)-5-chloro-α-(cyclopropylethynyl)-2-amino-α-(trifluoromethyl) benzenemethanol (**Figure 2**). The preparation of this chiral intermediate has been reported either by resolution or by chiral reactions[8-13]. Major drawbacks of the reported methods include the use of costly resoluting reagents and costly chiral reagents. Therapeutic importance of the Efavirenz needs an efficient cost effective process to serve a major percentage of population in the developing countries.

Enzyme mediated reactions provide an important alternative to the chemical reactions in terms of cost and quality of the products[14,15]. Enzymes, especially lipases are known for their low cost and great tolerance towards their substrates. Lipase-catalyzed kinetic resolution of racemic compounds with various functionalities has become an important method for the preparation of enantiopure compounds. Among the resolution-based procedures, the enzymatic methods are emerging as a useful alternative. Lipase B from *C. antarctica* (Novozyme-435) has proven to be the most efficient catalyst for resolution reactions in organic solvents, allowing the preparation of a variety of optically active primary and secondary alcohols, primary amines and amides[16-22]. Environmentally benign character of the enzymatic processes is desirable for large scale industrial applications[23].

The commercial importance, along with structural complexity of the intermediate (*S*)-5-chloro-α-(cyclopropylethynyl)-2-amino-α-(trifluoromethyl)benzenemethanol (**Figure 2**) prompted us to undertake the synthesis of this intermediate enantiomer using enzymatic route. The strategy, to get the desired enantiomer, was the lipase catalyzed enantioselective acylation of the racemic compound (*R*/*S*)-5-chloro-α-(cyclopropylethynyl)-2-amino-α-(trifluoromethyl)benzenemethanol **1** using vinyl acetate as the acetylating agent. However, surprisingly, an unexpected benzoxazine derivative (Efavirenz analogue) was formed and herein the formation and characterization of the unexpected product 6-chloro-4-(cyclopropylethylnyl)-2-methyl-1,4-dihydro-4-(trifluoromethyl)-2*H*-3,1-benzoxazine **3** during the attempted enzymatic resolution of (*R*/*S*)-5-chloro-α-(cyclopropylethynyl)-2-amino-α-(trifluoromethyl) benzenemethanol **1** is reported.

## Results and Discussion

Different lipases *viz.* Novozyme-435, lypozyme, *Candida. rugosa* lipase (CRL) and porcine pancreatic

lipase (PPL) were screened for the enantioselective acylation of hydroxyl groups of racemic compound (*R*/*S*)-5-chloro-α-(cyclopropylethynyl)-2-amino-α-(trifluoromethyl) benzenemethanol **1** using vinyl acetate as the acylating agent in different solvents **Scheme I**. The initial attempts during the screening of the enzymes revealed that the conversion of starting material to an unknown product was observed in the case of Novozyme-435 and lypozyme as enzymes. Both Novozyme-435 and lipozyme were found to transform the starting material into the same product (as seen on TLC) upon incubation with vinyl acetate, however the rate of transformation was slower in the reaction catalyzed by Novozyme-435 than the reaction catalyzed by lipozyme. PPL and CRL did not catalyze the reaction and starting material was recovered unchanged in both the cases.

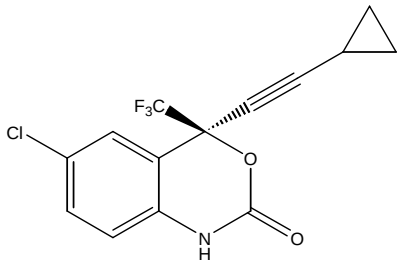



**Figure 1 —** Efavirenz (Sustiva™)

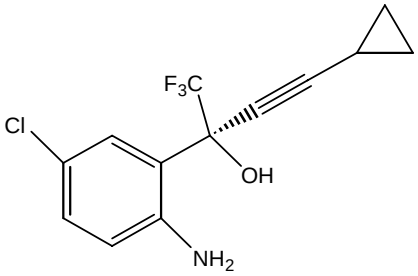
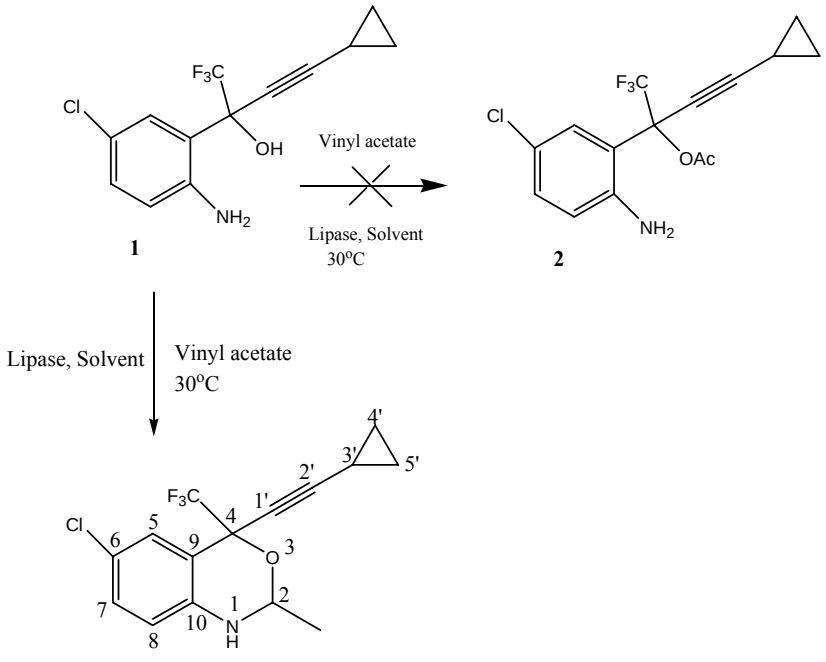



**Figure 2 —** Efavirenz intermediate

The product formed in reactions catalyzed by Novozyme-435 and lypozyme was isolated and subjected to the structural elucidation using $^{1}H$, $^{13}C$, DEPT, $^{1}H$-$^{1}H$ COSY and mass spectrometry and surprisingly, the spectroscopic data of the biotransformation product was not found to be in agreement with the expected structure **2** (**Scheme I)**. Based upon the detailed studies of its spectroscopic data, the product was identified as a novel, cyclized compound, 6-chloro-4-(cyclopropylethylnyl)-2-methyl-1,4-dihydro-4-(trifluoromethyl)-2*H*-3,1-benzoxazine **3**. In its $^{1}H$ NMR spectrum, a doublet at δ 1.38 integrating for three protons was assigned to the methyl group, which is further supported by a peak at δ 19.8 in its $^{13}C$ NMR spectrum. A multiplet in the range of δ 4.73-4.76 integrating for one proton was assigned to the C-2 proton, which was further supported by a peak at δ 76.6 in its $^{13}C$ NMR spectrum. The $^{1}H$-$^{1}H$ COSY experiments showed an interaction between C-2 proton and methyl protons and this further supported




**Scheme I** — Formation of cyclized product during the attempted acylation of Efavirenz intermediate **1** with lipase

**Table I** — Novozyme-435 catalyzed reaction of compound **1** with vinyl acetate at 30°C

| Entry | Solvent | Time (hr) | Yield[a] (%) |
|---|---|---|---|
| 1 | Tetrahydrofuran | 9.0 | 96.8 |
| 2 | Toluene | 7.5 | 95.7 |
| 3 | Hexane | 11.4 | 97.4 |
| 4 | Dioxane | 12.0 | 97.3 |
| 5 | Diisopropylether | 6.0 | 98.5 |
| 6 | Acetonitrile | 7.0 | 99.0 |

[a] Isolated yield

**Table II** — Lipozyme catalyzed reaction of compound **1** with vinyl acetate at 30°C

| Entry | Solvent | Time (hr) | Yield[a] (%) |
|---|---|---|---|
| 1 | Tetrahydrofuran | 7.0 | 95.0 |
| 2 | Toluene | 7.5 | 97.8 |
| 3 | Hexane | 13.0 | 96.3 |
| 4 | Dioxane | 12.3 | 94.0 |
| 5 | Diisopropylether | 3.0 | 98.0 |
| 6 | Acetonitrile | 4.0 | 98.2 |

[a] Isolated yield

the structure of the compound as 6-chloro-4-(cyclopropylethylnyl)-2-methyl-1,4-dihydro-4-(trifluoromethyl)-2*H*-3,1-benzoxazine **3**.

After establishing the structure of compound **3,** the attention was focused on the role of different solvents on the cyclization reaction and a series of reactions with Novozyme-435 as enzyme in different solvents at 30°C were performed and allowed the reaction till the complete conversion of the substrate **1** into the product 6-chloro-4-(cyclopropylethylnyl)-2-methyl-1,4-dihydro-4-(trifluoromethyl)-2*H*-3,1-benzoxazine **3** (**Scheme I**). The cyclization reaction affected by Novozyme-435 in DIPE completed in only 6 hr, whereas in dioxane, quantitative conversion occurred in 12 hr **(Table I)**.

We also investigated the role of different solvents on the cyclization reaction catalyzed by lypozyme at 30°C and as it was observed during initial screening of enzymes, the rate of transformation was found to be faster in the reaction catalyzed by lipozyme compared to the Novozyme-435 in most of the solvents (**Tables I** and **II**). The cyclization reaction in diisopropylether affected by lypozyme was found to be 2 times faster than the reaction catalyzed by Novozyme 435 in the same solvent, the reaction times for quantitative conversion of **1** into **3** with lypozyme and Novozyme-435 as enzymes were found to be 3 hr and 6 hr, respectively (**Tables I** and **II**).

We also performed the reaction under identical conditions but without addition of the enzyme, but it did not yield any product and it further confirmed that the formation of **3** from **1** occurs only in the presence of Novozyme-435 and lypozyme. We are currently trying to find out the mechanism for formation of 6-chloro-4-(cyclopropylethylnyl)-2-methyl-1,4-dihydro-4-(trifluoromethyl)-2*H*-3,1-benzoxazine **3** from **1** with vinyl acetate in the presence of Novozyme-435/lypozyme.

## Materials and Methods

The solvents used and vinyl acetate were products of Aldrich and used after distillation. The $^{1}H$ and $^{13}C$ NMR spectra were recorded on a Bruker AC-300 Avance spectrometer at 300 and at 75.5 MHz, respectively using TMS as internal standard. The chemical shifts values are on δ scale and the coupling constants (*J*) are in Hertz. The Novozyme-435, (CAL-B immobilized on accurel) was purchased from Novozymes A/S and used after storing in vacuuo over $P_2O_5$ for 30 hr. Analytical TLCs were performed on pre-coated Merck silica gel 60F254 plates. Silica gel (100–200 mesh) was used for column chromatography.

## Experimental Section

### Lipase catalyzed synthesis of 6-chloro-4-(cyclopropylethylnyl)-2-methyl-1,4-dihydro-4-(trifluoromethyl)-2*H*-3,1-benzoxazine 3

Novozyme-435/Lipozyme (100 mg) was added to the mixture of the racemic compound **1** (1 mmole) in an appropriate solvent (20 mL) and vinyl acetate (1 mmole). The reaction- mixture was incubated at 30°C in an incubator shaker and the progress of reaction was monitored by TLC. On completion of reaction, the reaction-mixture was diluted with corresponding solvent and the reaction was quenched by filtering off the enzyme. The organic solvent was evaporated under vacuuo and the residue was subjected to column chromatography using petroleum ether/ethyl acetate as eluent to afford the pure compound **3** in good yields (95 to 99%). It was obtained as a white solid, m.p. 150-52°C; $^{1}H$ NMR (300 MHz, DMSO-$d_6$): δ 0.65 (2H, m, cyclopropyl methylene), 0.83–0.88 (2H, m, cyclopropyl methylene), 1.38 (3H, d, $J$ = 5.49 Hz, C$H_3$), 1.44–1.49 (1H, m, C-3'H), 4.73-4.76 (1H, m, C-2H), 6.74 (1H, d, $J$ = 8.67 Hz, C-8H), 6.97 (1H, br s, NH), 7.17 (1H, s, C-5H) and 7.22 (1H, dd, $J$ = 2.40 Hz, $J$ = 6.29 Hz,

C-7H); $^{13}$C NMR (75.5 MHz DMSO-$d_6$): δ -1.13 (C-3'), 8.36 and 8.44 (C-4' and C-5'), 19.83 ($CH_3$), 70.30 (C-4), 73.3 (C-2'), 76.6 (C-2), 93.4 (C-1'), 117.8 (C-8), 121.3 (C-6), 121.5 ($CF_3$), 125.1 (C-9), 125.6 (C-5), 129.5 (C-7) and 143.0 (C-10); HRMS: *m*/*z* Calcd for $C_{15}H_{13}ClF_3NO$ 315.0638, Found 315.0640.

## Conclusion

The formation of an benzoxazine derivative during the attempted lipase catalyzed synthesis of (*S*)-5-chloro-α-(cyclopropylethynyl)-2-amino-α-(trifluoromethyl) benzenemethanol in the present study has revealed the capability of Novozyme-435 and lypozyme towards the cyclization of amino alcohols and its derivatives for synthesis of Efavirenz analogues and other benzoxazine derivatives.

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