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Research Article

STEREOSELECTIVE ENZYMATIC SYNTHESES OF ANGIOTENSIN CONVERTING ENZYME INHIBITOR DRUGS

V .V. S. V. PRASAD[1], P. RAMA RAO[2], A. NAIDU[3], P. VENKATESWAR RAO[4]

For author affiliations, see end of text

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ABSTRACT

Angiotensin converting enzyme (ACE) inhibitor drug intermediates, ECPPA (S)-ethoxycarbonyl-3-phenylpropyl) L-alanine) **,**ECPPL(N^2-(1(S) ethoxycarbonyl-3 Phenyl propyl)- N^6 trifloro acetyl L-lysine)have been synthesized by using the enzyme Lipase. Angiotensin converting enzyme inhibitor drug intermediates are synthesized stereo selectively by using Novo SP 435 supplied by Novozymes. Choral specificity for the ACE inhibitor drug intermediates is (SS). The formed desired (SS) isomer is matched by the standard sample with HPLC method.

KEYWORDS

Angiotensin converting enzyme (ACE) inhibitor drug intermediates, ECPPA (N^2 (1(S)-ethoxycarbonyl-3-phenylpropyl) L-alanine) and ECPPL (N^2-(1(S)-ethoxycarbonyl-3 Phenyl propyl) - N6 trifloro acetyl L-lysine)

INTRODUCTION

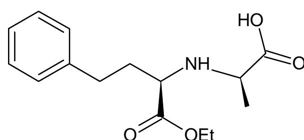
Enzymes are remarkable molecular devices that determine the pattern of chemical transformations in organic synthesis. The most striking characteristics of enzymes are their catalytic power and stereo specificity. Enzymes are highly effective in catalyzing diverse chemical reactions because of their ability to specifically bind to a substrate and their ability to accelerate reactions by several orders of magnitude. Applying enzymes or organisms in organic synthesis has become one of the fastest growing techniques for the development of new methodology and the work in this area has been comprehensively growing every year[1-3]. Lipase has been utilized by many scientists for highly stereo specific syntheses of chiral compounds.[4] Angiotensin-converting enzyme inhibitors [5](ACE inhibitors) are a new class of potent specific inhibitors which generate the powerful vasoconstrictor angiotensin. Angiotensin-converting enzymes[6] (peptidol dipeptide hydrolase. EC 3.4.15-1) generates the powerful vasoconstrictor substance angiotensin II by removing the C-terminal dipeptide from the precursor decapeptide angiotensin.I) [7]. The enzyme also inactivates the vasodilating substance bradykinin[8] Angiotensin-converting enzyme (ACE) inhibition is an effective therapy for the control of hypertension and congestive heart failure. Angiotensin Converting Enzyme (ACE) Inhibitors are used to manage hypertension (high blood pressure[9-10]. These medications lower blood pressure by preventing the formation of angiotensin II, a hormone in the body that causes blood vessels to constrict (close)[11] This constriction of the blood vessels is due to angiotensin II, a hormone that is an important component of hypertension. Blocking the hormone causes the blood vessels to relax or dilate (open), and thus helps to reduce blood pressure.[12]

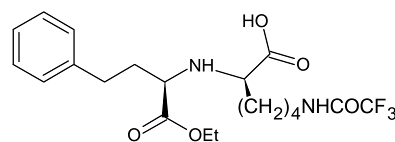
Angiotensin-converting enzyme inhibitor (ACE inhibitors) drugs are Benazepril [13] Lotensin[14]Captopril[15] (Capoten), Enalapril (Vasotec), Enalaprilat [16] Injection (Vasotec IV) Fosinopril [17] Lisinopril [18] Prinivil, Zestril, Moexipril[19] Univasc, Perindopril[20] Aceon, Quinapril Cupric, Ramipril [21] Altace, Trandolapril [22]. Enalapril, Ramipril, Trandalapril, Quinopril and Lisinopril have been synthesized. Syntheses of Enalapril, Quinapril, Ramipril and Trandolapril require Common building block Intermediate like N^2 (S) Ethoxy carbonyl phenyl propyl alanine (ECPPA). Syntheses

of Lisinopril require the intermediate N^2 – ((S)-1 ethoxy carbonyl-3-phenyl propyl)-trifloroacetyl L-lysine (ECPPL).

Various synthetic methods are reported for the preparation of ECPPA and ECPPL [23]. A favorable diastereoselective synthesis of - (1-S-ethoxycarbonyl-3-phenylpropyl)-S-alanine is carried out by Michael addition of S-alanine benzyl ester to ethyl-4-oxo-4-phenyl-2-butenoate in a region and diastereo selective fashion and subsequent catalytic hydrogenolysis.

In the preparation of ECPPA, the carboxylic acid group of alanine is first protected by benzyl group and condensed with acryl ate in presence of mild base like TEA giving ECPPA [24]. ECPPL is prepared from Ph CO CHCH CO_2Et when reacted with CF_3 CONH $(CH_2)_4$ CH (NH_2) COOH in presence of an alkali metal salt to form a Michael addition product followed by the addition of not less than one equivalent of mineral acid and after completion of hydrogenation, the desired product is obtained. Acid addition is to prevent conversion of SS isomers to RS isomer [25]. Another process for the preparation of ECPPL is the condensation of β phenyl propionaldehyde with N^6 trifloro acetyl L- Lysine in presence of NaCN, which after hydrolysis gives the SS isomer. After purification it gives 99% of chiral purity product [26].





ECPPA

N^2-1(S)-ETHOXYCARBONYL-3-PHENYLPROPYLL-ALANINE

ECPPL

N^6 (1(S)-ETHOXYCARBONYL-3-PHENYLPROPYL)-TRIFLUOROACETYLL- LYSINE

Fig I: STRUCTURES OF ECPPA AND ECPPL

EXPERIMENTAL PROCEDURE

All chemicals used were of commercial grade. Here we describe a stereo selective synthesis of the generally usable component for the ACE inhibitors i.e.N^2-(s)-1-ethoxy carboxyl-3-phenyl propyl)-L-alanine and further application of N^2 –(s)-1-ethoxy carboxyl-3-phenyl propyl)-N^6 tri floro acetyl L-lysine. (Fig I)The reaction of trans beta Benzoyl Acryl ate with different amino acids L-alanine or tri floro acetyl L-Lysine in presence of Novo SP 435 led to the corresponding Michael addition product. The products produced from the enzymatic condensation are analyzed by HPLC method .In order to quantify their concentration and reaction rate HPLC method is quite useful. HPLC conditions are useful for the reaction monitoring and standard sample retention time comparisons. (Table1).We have conducted various experiments with different solvents like Diisopropyl ether, Diethyl ether, Tert-butyl alcohol, 1,4 Dioxane, Toluene, Benzene, N-Hexane, Acetonitrile, Ethyl acetate and the substrate and enzyme ratio has been used as 100:1(Table 2& Table 3).

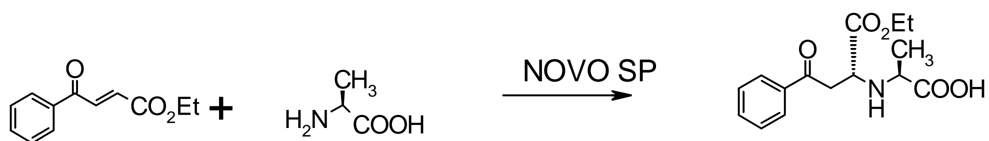
Reaction was conducted below room temperature and the products formed were confirmed by HPLC reaction monitoring. The samples were collected from reaction bottle and are acidified with mineral acid and then they are analyzed. We observed that minimum time for the optimum conversion of reactants into products is 6 to 7 hours. If the reaction is kept longer than 7 hrs some side products have been formed. These are mainly formed from theβ-Benzoyl acryl ate. The positive aspect of the synthesis of ECPPA and ECPPL is with enzymatic reaction. The highlight of the reaction is the formation of desired (S, S) configuration of ECPPA and ECPPL and there were no unwanted (R.S) configuration products. When L-lysine derivative is condensed with β-Benzoyl acryl ate in presence of LiOH, it forms both SS: RS configuration products in the ratio of 70:30. Novo SP 435 enzyme is highly specific in its biocatalysis in the synthesis of ACE inhibitor intermediates. It catalyses the reaction in such a way that it avoids the formation of unwanted (R, S) isomers and forms the desired drug product of (S, S) isomer and the difficulties in the separation of the desired isomer are avoided. It is a much easier synthetic preparation than the conventional organic synthesis. It is an advantageous synthesis over the conventional

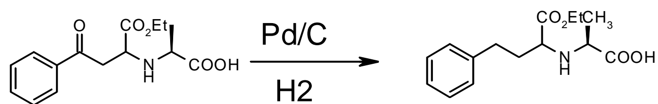
synthesis. The method can be considered as a biotechnological synthesis. It adds to the protection of the environment and can be classified under green chemistry experiment.Percent of Michael addition product formed with Novo SP 435 in various organic solvents are given as under. (Table 2 & 3)

TYPICAL EXPERIMENTAL PROCEDURE

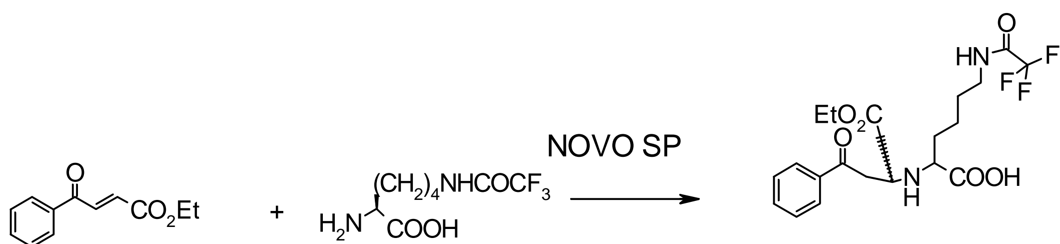
A round bottom flask is taken. It is fitted with a magnetic stirrer. Novo SP 435 enzyme and required amount of the solvent (10 volumes of the solvent) were added. β-Benzoyl acryl ate and L- Alanine are taken in equal molar ratios at 25 ^{o}C .The reaction mass is maintained for 5 to 6 hours and the samples were analyzed as per the procedure shown in the above HPLC methods (Table-1). The samples were analyzed regularly for every one hour .All the samples were compared with standard samples prepared from the standard procedures. The reactions were run for two to three times and the reproducibility of the reaction was checked. (Scheme I). The reaction was conducted with various solvents as shown in table-2.In the preparation of

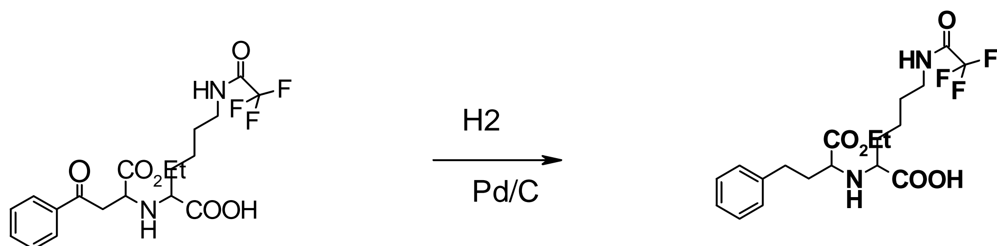
ECPPL β-Benzoyl acryl ate and Tri floro acetyl L-lysine were taken in the ratio of 1:1 at 25°C in a round bottom flask fitted with a magnetic stirrer. Novo SP 435 enzyme was taken in the flask and it was dissolved in ten volumes of the solvent. The reaction mixture was stirred for 7 to 8 hrs. (Scheme II). The samples were analyzed as per the HPLC methods shown in table-1. The samples were analyzed regularly for every hr. The samples were compared with the standard samples .The reproducibility of the reaction was checked number of times by repeating the experiment. The reaction was conducted with various solvents and the results were shown in table-3.





Scheme – I





Scheme – II

METHODS AND MATERIALS

Analytical data were obtained with a Thermo Finnigan Flash EA 1112 analyzer. ^{1}H NMR spectra were obtained at 300 MHz using a Brucker 300 Avance NMR spectrometer running X-WIN NMR software. The chemical shifts are relative to tetramethylsilane (TMS). All chemicals and solvents were reagent grade and were used as purchased without further purification. Melting points were determined using an Electro-thermal 9100 melting-point apparatus. FT-IR spectra were recorded as KBr pellets on a Jasco FT/IR-600 Plus spectrometer. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectra were recorded at ambient temperature in $CDCl_3$. Chemical shifts (d) are expressed in units of parts per million relative to TMS.

RESULTS AND DISCUSSION

Stereo selective enzymatic synthesis of ACE inhibitor drug intermediateN^2 (1(S)-ethoxycarbonyl-3-phenylpropyl) L-alanine) ECPPA, (N^2- (1(S)-ethoxycarbonyl-3 Phenyl propyl) - N6 trifloro acetyl L-lysine) ECPPL has been carried out. Vicente Gotor [27] has reported the mechanism of Lipase biocatalysed Michael addition of secondary amines to acrylonitrile using Novozyme 435. Enzymatic desymetrization of the homo allylic diol with Novo SP 435 allowed optical pro(s) selectivity to provide the desired (-) (s) monoacetate. The predominant Enantio pure monoester produced by ammonolysis of di ethyl 3 hydroxy glutarate catalyzed by immobilized lipase B from (Novozyme 435) was ethyl (3S)-4-carbamoyl- 3 hydroxy butanoate. In the present strategy, the synthesis of ACE inhibitor drug intermediate likes ECPPA and ECPPL were carried out by Novo SP 435 supplied by Novozymes. In this synthetic procedure, the required chiral specificity for the above intermediates is SS and the chiral purity of the product was observed to be high (>99%).

Here we reported an unprecedented NOV SP 435 catalyzed condensation of β-benzyl acryl ate with amino acid derivatives. Different amino acid derivatives with β benzoyl acryl ate was prepared during the preparation of ACE inhibitor drug intermediates. The catalytic effect of the enzyme was demonstrated by the

combination of different experiments. These experiments demonstrated that state of the art enzymatic transformation has reached an extraordinary level making them valuable and innovative methods for use in the chemical and pharmaceutical industry. The synthesized desired (SS) isomer has been matched by the standard sample with known HPLC methods. Details of HPLC column are given in table 1. The analytical data of the products is given in table 4.

Table-1

S.NO.	CONDITIONS	ECPPA	ECPPL
1	Column	Finepaksil C18-5	Finepaksil C18-5
2	Column Temperature	40^0 c	40^0 c
3	Flow Rate	1.5 ml/Minute	1 ml/Minute
4	Detector	UV 210nm	UV 210nm
5	Eluent	60mM phosphate Buffer (ph 2.5)/acetonitrile 85/15	60mM phosphate buffer (ph 2.5)/ Acetonitrile 85/15

Table -2: Michael addition reaction of ECPPA with different solvents.

S.No.	Subatrate-1	Subatrate-2	Solvent	Michael addition product Name	% Of Michael addition product formed by HPLC
1.	β-Benzoyl acryl ate	L-Alanine	Diisopropyl ether	ECPPA	78
2.	β-Benzoyl acryl ate	L-Alanine	Diethyl ether	ECPPA	68
3.	β-Benzyl acryl ate	L-Alanine	Tert-butyl alcohol	ECPPA	65
4.	β-Benzoyl acryl ate	L-Alanine	1,4 Dioxane	ECPPA	63
5.	β-Benzoyl acryl ate	L-Alanine	Toluene	ECPPA	60
6.	β-Benzoyl acryl ate	L-Alanine	Benzene	ECPPA	55
7.	β-Benzoyl acryl ate	L-Alanine	N-Hexane	ECPPA	52
8.	β-Benzoyl acryl ate	L-Alanine	Acetonitrile	ECPPA	48
9.	β-Benzoyl acryl ate	L-Alanine	Ethyl acetate	ECPPA	29

Table-3: Michael addition reaction of ECPPL with different solvents.

S.No.	Subatrate-1	Subatrate-2	Solvent	Michael addition product name	% Of Michael addition product formed by HPLC
1.	β-Benzoyl acryl ate	Tri floro acetyl L-lysine	Diisopropyl ether	ECPPL	76
2.	β-Benzoyl acryl ate	Tri floro acetyl L-lysine	Diethyl ether	ECPPL	68
3.	β-Benzoyl acryl ate	Tri floro acetyl L-lysine	Tert-butyl alcohol	ECPPL	65
4.	β-Benzoyl acryl ate	Tri floro acetyl L-lysine	1,4 Dioxane	ECPPL	61
5.	β-Benzoyl acryl ate	Tri floro acetyl L-lysine	Toluene	ECPPL	58
6.	β-Benzoyl acryl ate	Tri floro acetyl L-lysine	Benzene	ECPPL	55
7.	β-Benzoyl acryl ate	Tri floro acetyl L-lysine	n-Hexane	ECPPL	53
8.	β-Benzoyl acryl ate	Tri floro acetyl L-lysine	Acetonitrile	ECPPL	40
9.	β-Benzoyl acryl ate	Tri floro acetyl L-lysine	Ethyl acetate	ECPPL	22

IR spectral data of ECPPA

IR spectrum of ECPPA is recorded in KBr (Pellets). The FT IR spectrum shows characteristic peaks at 3400-3600 cm^{-1} region, which is characteristic frequency of νOH of L alanine. A stretching frequency observed at 1735 cm^{-1} is due to ν C=O frequency of ester group. A ν C=O at 1710 cm^{-1} is due to carboxylic acid group. A ν CH stretching vibration observed at 2850 cm^{-1} is due to CH_3 and CH_2 groups. The νOH bending vibration is observed at 1400 $cm^{-1.}$ The νNH stretching vibration observed at 3300 cm^{-1} is a weak absorption. An absorption band observed at760 cm^{-1} is interpreted to the wagging band of νNH .

^{1}H NMR Spectral Data of ECPPA

The ^{1}HNMR spectrum of ECPPA was recorded in $CDCl_3$ using TMS as the standard. The spectrum shows its characteristic signals accounting for the proposed structure of the compound. A signal at δ 1.23 is due to CH_3 (a) (s, 3H, CH_3 (a)). A signal at δ 1.30 is due to the CH_3 of C_2H_5 (s, 3H, CH_3 (b)) of COOEt. A signal at δ 2.09 is due to CH_2 (s, 2H, of CH_2). Another signal at δ 2.55 is due to (s, 2H, CH_2 adjacent to phenyl ring). A signal at δ 3.45 is due to the tertiary carbon attached to NH group(s, 1H, CH of NH group). A signal at δ 3.67 is due to another tertiary carbon attached to COOH group (s, 1H, CH of COOH group). A signal observed at δ 4.12 is due to CH2 of C_2H_5 (s, 2H, CH_2 of C_2H_5). A cluster of signals observed in the region of 7.08-7.21 are due to aromatic protons .A signal at δ 11.00 is due to hydroxyl proton (s.1H, OH).

^{13}C NMR Spectral Data of ECPPA

The ^{13}C NMR spectrum of ECPPA was recorded in $CDCl_3$ using TMS as standard. A signal at δ 14.1 is due to C of CH_3 of C_2H_5 of COOEt group. A signal of δ 17.4 is due CH_3 of L –alanine moiety. A signal at δ 32.9 is due to C of CH_2 group. A signal at 30.6 is due to C of CH_2 attached to phenyl group. A signal at δ

57.6 is due to asymmetric carbon of L alanine moiety. A signal at δ 58.2 is due to asymmetric carbon of phenyl propyl group. A signal at δ 61.3 is due to C of CH_2 group of COOEt group. Signals at δ 126.1, 128.2 and 128.9 are due to aromatic carbons of phenyl group. A signal at δ138.1 is due to the tertiary aromatic carbon of phenyl group attached to propyl group. A signal at 171.6 is due to carbonyl carbon of COOEt group. A signal at δ 174.9 is due to the carbonyl carbon of carboxylic acid group.

IR Spectral Data of ECPPL

IR spectrum of ECPPL was recorded in KBr (Pellets). The FT IR spectrum shows its Characteristic peaks at 3200-3600 cm^{-1} is due to the characteristic νOH. A stretching frequency observed at 1725 cm^{-1} is due to ν C=O frequency of ester group. A ν C=O at 1706 cm^{-1} is due to νC=O of carboxylic acid group. The νC=O of CF_3-C=O is observed at 1680 cm^{-1}. A ν CH stretching vibration observed at 2850 –2950 cm^{-1} is due to CH_3 and CH_2 groups. The νOH bending vibration is observed at 1420 cm^{-1}. The stretching vibration observed at 3250 -3300 cm^{-1} as a weak absorption is due to νNH. The νNH wagging absorption band is observed at 760 -780 cm^{-1} [28].

^{1}H NMR spectrum of ECPPL

The ^{1}H NMR spectrum of ECPPL is recorded in $CDCl_3$ using TMS as standard (δ) (ppm). A signal at δ 1.3 is due to CH_3 group (s, 3H, CH_3); Signals from 1.2 to 2.5 are due various CH_2 protons. These signals account for seven different CH_2 groups present in the molecule. A signal at δ 2.01 is due to the CH proton of the asymmetric carbon present on the phenyl propyl group. A signal at δ 3.49 is due to the asymmetric CH group of alanine moiety. Signals at 7.08, 7.12 and 7.21 are due to the aromatic protons on the phenyl group. A signal at δ 8.0 is due to the NH proton. A signal at δ 11.0 is due to the hydroxyl proton of carboxylic acid group.[29]

^{13}C NMR spectrum of ECPPL

A ^{13}C NMR spectrum of ECPPL was recorded in $CDCl_3$ using TMS as standard. The ^{13}CNMR spectrum shows its characteristic NMR signals correlating very well with the expected structure of the molecule. A signal at δ 14.1 is due to methyl carbon of $COOC_2H_5$ group. Signals at δ 21.4, 29.6, 29.8, 31.2, and 33.4 are due to Carbons of CH_2 group. A signal at δ 40.1 is due to the methylene carbon attached to the NH group. A signal at δ 61.0 is due to the methylene carbon attached to the asymmetric carbon of phenyl propyl group. Signals at 63.9 and 58.7 are due the asymmetric carbons. A cluster of signals at 126.1, 128.2 and 128.9 are due to the aromatic carbons of phenyl group. A signal at δ 138.1 is due to the tertiary carbon of the phenyl group attached the propyl group. A signal at δ 116.3 is due to the carbon of CF_3 group. A signal at δ 157.4 is due to the carbonyl carbon attached to the trifloro methyl group. A signal at δ 170.3 is due to the carbonyl carbon of the COOEt group. A signal at δ 174.9 is due to the carbonyl group of carboxylic acid group of amino acid moiety.

Table 4: Analytical Data of the compounds

Name	Formula	Found(Calcd) Carbon	Hydrogen	Nitrogen
(R) –2-((S) – 4-Oxo-4-phenylburan-2-ylamino)propionic acid(Pro drug)	$C_{15}H_{19}NO_5$	61.50 (61.42)	6.60 (6.53)	4.85 (4.78)
ECPPA	$C_{15}H_{21}NO_4$	65.00 (64.50)	7.71 (7.58)	4.88 (5.01)
(R)-2-((S)-4-oxo-4-phenyl-butan-2yl-amino)-6-(2, 2, 2)-trifloro acetamido) hexanoic acid(Pro drug)	$C_{20}H_{25}F_3N_2O_6$	54.10 (53.81)	5.71 (5.64)	6.50 (6.28)
ECPPL	$C_{20}H_{27}F_3N_2O_5$	56.10 (55.50)	6.41 (6.29)	6.53 (6.42)

ACKNOWLEDGEMENTS

The authors are thankful to Principal, Nizam College, Hyderabad for providing laboratory facilities and for permitting to record spectral data.

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CURRENT AUTHOR ADDRESSES:

1: Stereo Drugs, Prashanthi Nagar, Kukatpally, Hyderabad Pin-500072, A.P, INDIA.

2: UPM pharmaceuticals Inc, 6200, Seaforth, Baltimore MD 21224, USA

3: Department of Chemistry, JNTU College of Engineering Kukatpally, Hyderabad-500072, INDIA.

4. Department of Chemistry, Nizam College, (O.U.) Hyderabad – 500 001.

Corresponding Address:

VVSV Prasad, Stereo Drugs,

Plot No-79, H.NO 5-5-35/152/1

First Floor, Prashanthi Nagar,

Kukatpally, Hyderabad-72

Mail: prasadvvsv@Rediffmail.com, valivarthip@Yahoo.com