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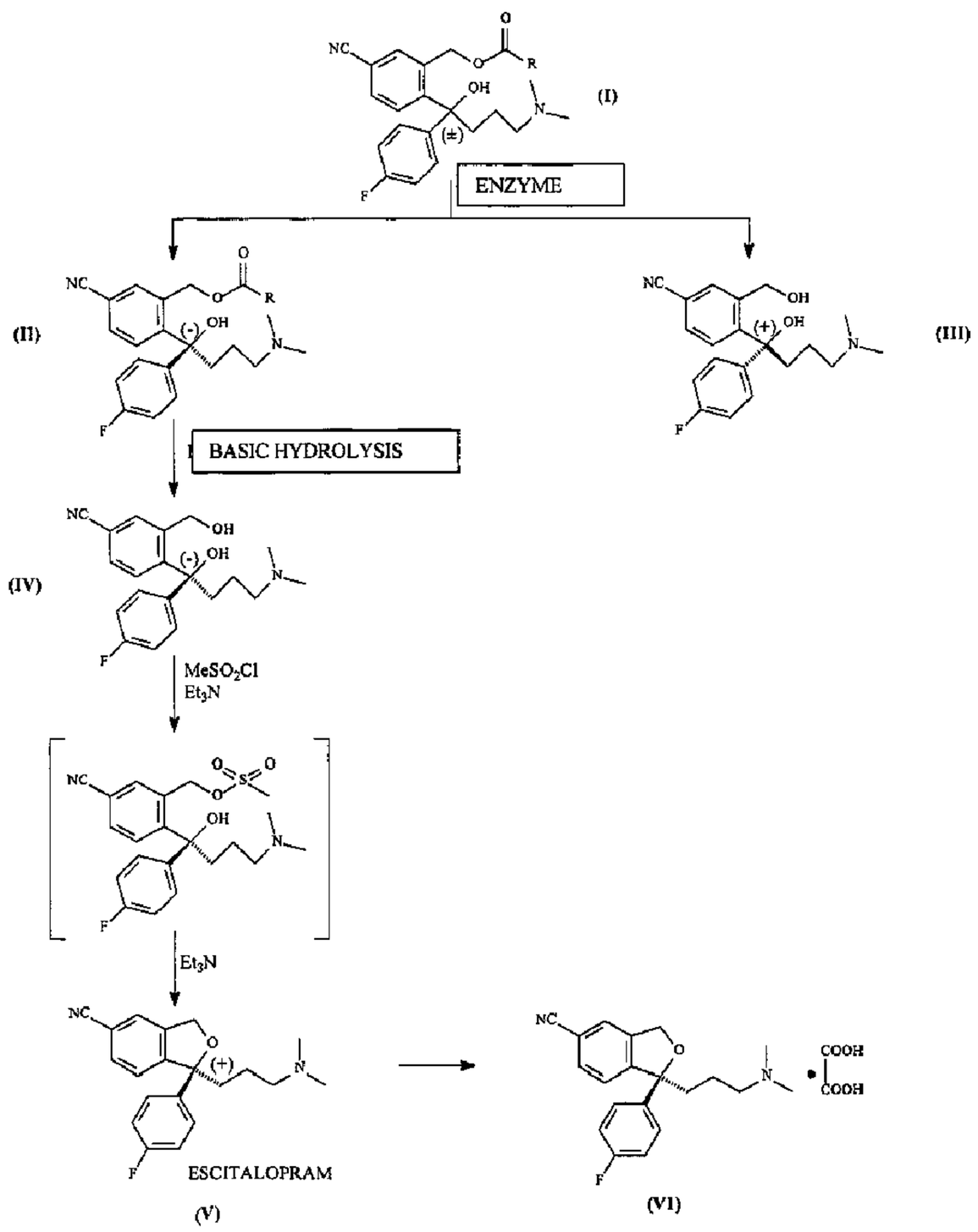
(71) Demandeur/Applicant:
ADORKEM TECHNOLOGY SPA, IT

(72) Inventeurs/Inventors:
COTTICELLI, GIOVANNI, IT;
ROCCHIETTI, SILVIA, IT;
TERRENI, MARCO, IT;
PREGNOLATO, MASSIMO, IT;
SALVETTI, RAUL, IT

(74) Agent: RICHES, MCKENZIE & HERBERT LLP

(54) Titre : PROCEDE CHIMIO-ENZYMATIQUE POUR LA PREPARATION D'ESCITALOPRAM
(54) Title: CHEMO-ENZYMATIC PROCESS FOR PREPARING ESCITALOPRAM



(57) Abrégé/Abstract:
A process is described for preparing the intermediate for synthesizing escitalopram and the pharmaceutically acceptable salts thereof from 4-(4-dimethylamino)-1-(4'-fluorophenyl)-1-(hydroxybutyl)-3(acyloxymethyl) benzonitrile which, by means of enzymatic enantiomeric resolution, is synthesized into one of the enantiomers thereof.

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(71) Applicant *(for all designated States except US)*: **ADORKEM TECHNOLOGY SPA** [IT/IT]; Via L. Da Vinci, 28, I-24062 Costa Volpino (IT).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **COTTICELLI, Giovanni** [IT/IT]; Via Penati 8, I-20063 Cernusco sul Naviglio (IT). **ROCCHIETTI, Silvia** [IT/IT]; Via Madonna di Re, 2, I-28845 Domodossola (IT). **TERRENI, Marco** [IT/IT]; Via Bramante, 40, I-20154 Milano (IT). **PREGNOLATO, Massimo** [IT/IT]; Via della Stazione 80, I-27020 Carbonara al Ticino (IT). **SALVETTI, Raul** [IT/IT]; Via 24 Maggio, 7, I-25040 Malonno (IT).

(74) Agent: **PISTOLESI, Roberto**; Dragotti & Associati Srl, Via Turati, 32, I-20121 Milano (IT).

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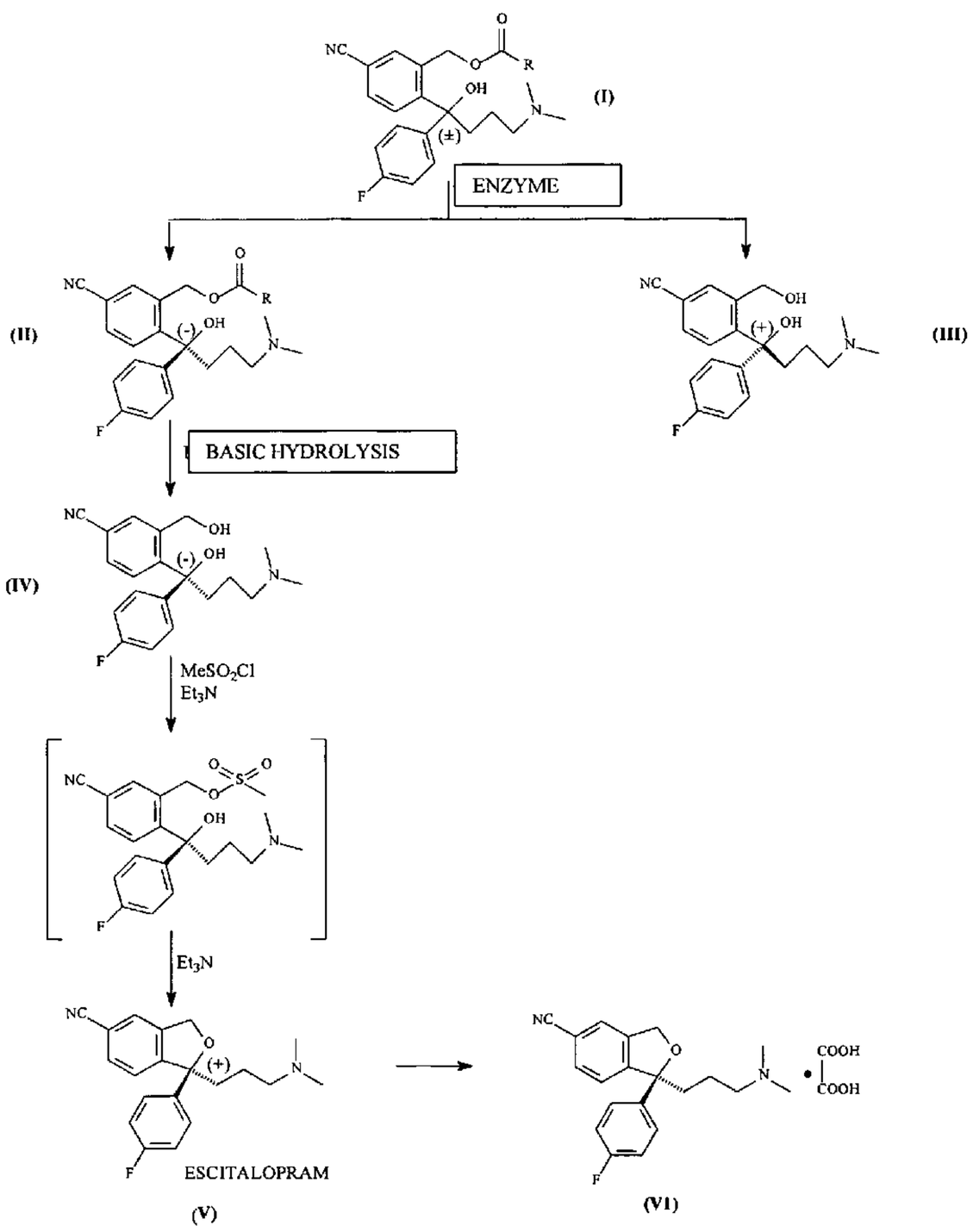
[Continued on next page]

(54) Title: CHEMO-ENZYMATIC PROCESS FOR PREPARING ESCITALOPRAM



(57) Abstract: A process is described for preparing the intermediate for synthesizing escitalopram and the pharmaceutically acceptable salts thereof from 4-(4-dimethylamino)-1-(4'-fluorophenyl)-1-(hydroxybutyl)-3(acyloxymethyl) benzonitrile which, by means of enzymatic enantiomeric resolution, is synthesized into one of the enantiomers thereof.

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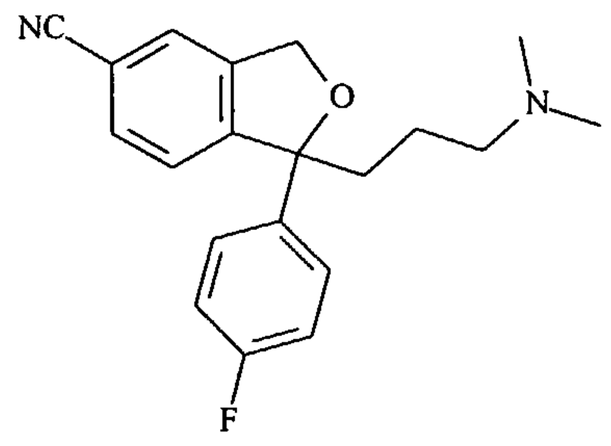
CHEMO-ENZYMATIC PROCESS FOR PREPARING ESCITALOPRAM

The present invention relates to a process for preparing enantiomerically pure 1-(3-dimethylaminopropyl)-1-(4'-fluorophenyl)-1,3-dihydroisobenzofuran-5-carbonitrile.

The above-mentioned compound, whose structural formula is set out below,



is a known active ingredient, better known as "citalopram", which is used for preparing pharmaceutical compositions which are intended for the treatment of depression.

Citalopram was described for the first time in Belgian patent application BE850401 (and in the corresponding American patent US 4,136,193); a number of patent documents further relate to methods for its preparation.

Being provided with a chiral centre, citalopram is generally produced and marketed in the form of a racemic mixture.

As set out in EP347066, the S(+) enantiomer, better known as escitalopram, is responsible for practically the whole of the pharmacological activity of racemic citalopram. European patent application EP347066 substantially describes two methods for preparing escitalopram.

The first method takes as a basis racemic 4-(4-dimethylamino)-1-(4'-fluorophenyl)-1-(hydroxybutyl)-3-(hydroxymethyl)benzonitrile which is subsequently esterified with an enantiomerically active acyl chloride, such as (+) or (-)-α-methoxy-α-trifluoromethylphenylacetyl chloride. From each (+) and (-) acyl chloride, there are obtained 2 diastereoisomeric esters which are separated by

means of HPLC, thus obtaining an enantiomerically pure ester; subsequent cyclization in the presence of potassium t-butoxide in toluene allows the pure enantiomer of citalopram to be obtained from each ester.

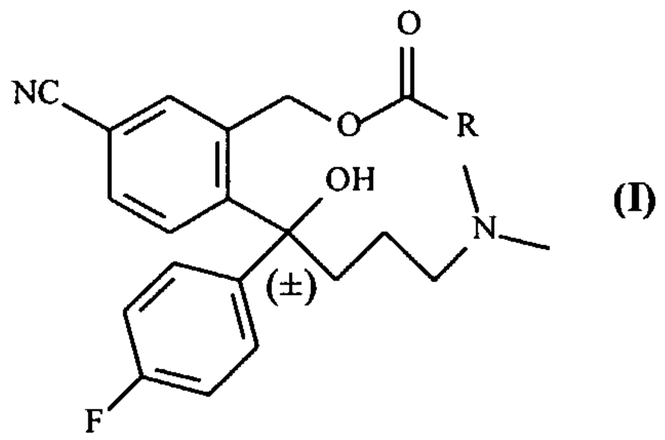
The second method takes as a basis enantiomerically (for example, (+)) pure 4-(4-dimethylamino)-1-(4'-fluorophenyl)-1-(hydroxybutyl)-3-(hydroxymethyl)benzonitrile. In order to obtain that enantiomerically pure product, the amine is salified with an enantiomerically active acid, such as, for example, tartaric acid, in order to provide two diastereoisomeric salts which can be separated by crystallization. The pure enantiomer which is released from its salt is esterified to form a particularly labile ester (for example, with methane sulphonyl chloride) which, with the use of strong organic bases (for example, triethylamine), allows enantiomerically pure citalopram to be obtained.

Other methods for preparing escitalopram are described, for example, in American patent US6365747, in American patent application US2003/0060641, and in international patent applications WO03/000672, WO03/006449 and WO03/051861.

However, the above-described methods are characterized by the use of enantiomerically active acids and/or diastereoisomeric separations by crystallization or by means of HPLC, which set limits in terms of scalability of the process and reaction yields.

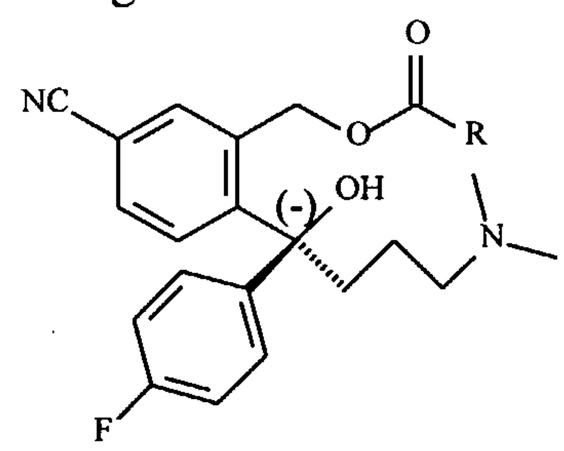
There has now been found a new process which allows the preparation of escitalopram with a high level of enantiomeric purity and without the disadvantages of the above-mentioned processes.

The process according to the present invention comprises resolution by the enzymatic route by means of an esterase from *Aspergillus niger* of the racemic mixture of a compound having formula I



(I)

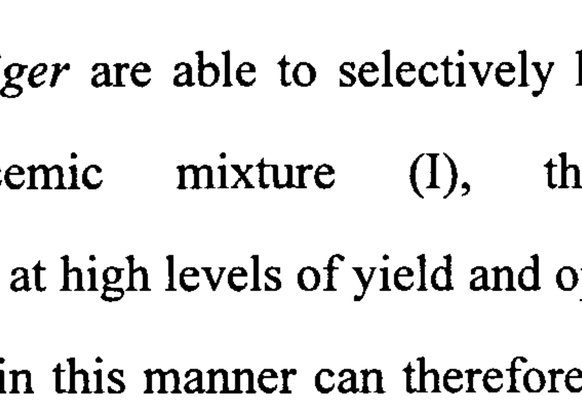
where R represents a C_1-C_4 alkyl radical or an aryl radical in order to provide the corresponding (-) enantiomer having formula II.



(II)

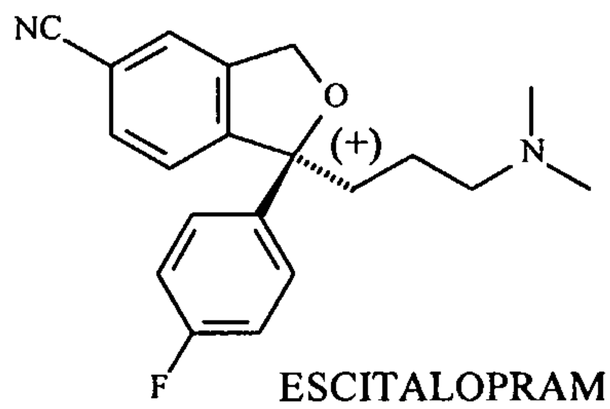
In fact, it has surprisingly been found, and constitutes the main subject-matter of the present invention, that, unlike the esterases generally known in the art, esterases from *Aspergillus niger* are able to selectively hydrolyze solely the (+) enantiomer of the racemic mixture (I), thereby allowing the (-) enantiomer to be collected at high levels of yield and optical purity.

The (-) enantiomer obtained in this manner can therefore be converted by means of hydrolysis, preferably basic hydrolysis, into (-)4-(4-dimethylamino)-1-(4'-fluorophenyl)-1-(hydroxybutyl)-3-(hydroxymethyl)-benzonitrile having formula IV.



(IV)

This can then be converted into escitalopram having formula V



ESCITALOPRAM

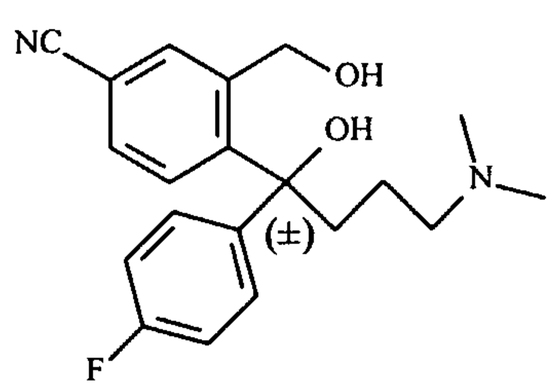
(V)

by means of condensation of the two hydroxyl groups using the methods known in the art, such as, for example, the method described in EP347066 (that is, processing with CH_3SO_2Cl in the presence of Et_3N), which is incorporated herein by reference.

The reaction diagram, comprising both the resolution and the conversion into escitalopram, is set out in Figure 1.

The racemic mixture of the compound having formula (I) can in turn be prepared according to methods known in the art.

For example, it can be prepared by following the instructions set out in EP171943, incorporated herein by reference. EP-171943 describes a synthesis method which provides for two consecutive Grignard reactions on the basis of 5-cyanophthalide; the first with 4-fluorophenylmagnesium bromide and the second with 3-(dimethylamino)propylmagnesium chloride on the magnesium derivative obtained in this manner in order to obtain a magnesium intermediate which, following acid hydrolysis, brings the precursor of citalopram to the diol having formula I'.



(I')

This intermediate is then acylated selectively on the hydroxymethyl in position 3 (of the benzonitrile) according to the methods known in the art, for example, by

reaction with the anhydride or the chloride of the corresponding acid.

According to a preferred embodiment, 4-(4-dimethylamino)-1-(4'-fluorophenyl)-1-(hydroxybutyl)-3-(hydroxymethyl)-benzonitrile is acetylated on the hydroxymethyl residue by using acetyl chloride. In this connection, there are used from 5-20 moles of acetyl chloride, preferably approximately 17 moles, per mole of starting product; the starting product is preferably added to the reaction medium whilst maintaining a preferred temperature of between 30 and 35°C; once the addition operations have been finished, the 4-(4-dimethylamino)-1-(4'-fluorophenyl)-1-(hydroxybutyl)-3-(acyloxymethyl) benzonitrile compound is readily isolated according to methods known in the art, for example, by evaporation at reduced pressure.

The resolution step is advantageously carried out in a solvent constituted by a mixture of an alcohol (preferably a C_1-C_4 alcohol, and even more preferably MeOH) and water, preferably at a proportion of from 0.5-1.5 to 1, even more preferably at a proportion of 1 to 1, effected at a preferred temperature of from 15-35° C, preferably between 20 and 25°C.

The water is advantageously used in the form of a phosphate buffer, preferably a monobasic potassium phosphate buffer.

The solvent is advantageously used at a quantity of from 3-5 litres, preferably from 3.5- 4 litres, per mole of substrate.

According to a preferred feature, the racemic compound having formula I is initially added to the solvent at a basic pH value, preferably approximately 8, and is subsequently brought to a value of 6.

The esterase enzyme from *Aspergillus niger,* preferably immobilized on resin, generally epoxy resin (Eupergit C), is then added and is advantageously used at a quantity of from 2500-3200 units, preferably from 2800-2900 units, per mole of substrate.

The resolution reaction is monitored by means of HPLC and allowed to continue until a hydrolysis yield of 55% is reached, which level is normally reached after approximately from 70-80 hours; then, after filtration, extraction is carried out using ethyl acetate as the preferred solvent and, after subsequent evaporation and suitable crystallization using a mixture of diethyl ether/ethyl acetate, there is obtained solely the (-)4-(4-dimethylamino)-1-(4'-fluorophenyl)-1-(hydroxybutyl)-3-(acyloxymethyl) benzonitrile.

Starting from this intermediate with reference to patent EP347066, it is possible, after hydrolysis and subsequent termination of the cycle, to obtain solely the escitalopram, obtained as a free base (**V**) or in the form of an oxalate salt (**VI**).

For the purposes of the present invention, the terms "racemic mixture", "racemate" and "racemic compound" are intended to refer not only to a 50:50 mixture of the two individual enantiomers, but also a mixture in which one of the two enantiomers is present in excess with respect to the remaining enantiomer.

The examples below are intended purely by way of illustration and must not be considered to limit the invention.

Example 1: Synthesis of 4-(4-dimethylamino)-1-(4'-fluorophenyl)-1-(hydroxybutyl)-3-(acetoxymethyl) benzonitrile

58.7 g of 4-(4-dimethylamino)-1-(4'-fluorophenyl)-1-(hydroxybutyl)-3-(hydroxymethyl) benzonitrile were placed in a 4-neck flask in a water bath at between 30 and 35°C, preferably 35°C, and 210 ml of acetyl chloride (17 moles per mole of starting product) were added dropwise into this medium. The admixture was left under agitation for 5 minutes, transferred to a 1-neck flask and evaporated at reduced pressure. There were obtained 79.02 g of 4-(4-dimethylamino)-1-(4'-fluorophenyl)-1-(hydroxybutyl)-3-(acetoxymethyl) benzonitrile in the form of an orange oily residue. ^{1}H NMR(DMSO-d6) δ 7.9 (d, 1H), 7.8 (d, 1H), 7.75 (s, 1H), 7.2 (d,2H), 7.1 (d, 2H) 6.2 (s, 1H), 5.2 (d, 1H),

4.8 (d, 1H), 3.0 (m, 2H), 2.60 (m, 6H), 2.3 (s, 2H), 1.9 (s, 3H), 1.7 (m, 1H), 1.4 (m, 1H).

Example 2: Enzymatic resolution of 4-(4-dimethylamino)-1-(4'-fluorophenyl)-1-(hydroxybutyl)-3-(acetoxymethyl) benzonitrile

10 g of 4-(4-dimethylamino)-1-(4'-fluorophenyl)-1-(hydroxybutyl)-3-(acetoxymethyl) benzonitrile (250 mM) were dissolved in 52 ml of MeOH, to which 52 ml of a 25 mM, pH8 monobasic potassium phosphate buffer were subsequently added.

The pH was then brought to 6 by carrying out suitable modifications with 2N HCl and compensating for the volume of HCl added with the same amount in ml of MeOH in order not to change the composition of the solution. The temperature of the solution was controlled so as to be in the order of between 20 and 25°C.

Finally, there were added approximately 75 units of esterase enzyme derivative which is obtained from crude lipase extract from *Aspergillus niger* and which was immobilized on epoxy resin, such as Eupergit C, according to conventional processes.

The reaction was carried out using an automatic titrator so as to keep the pH constant and was monitored by means of HPLC until a hydrolysis level of 55% (from 70-80 hours) was obtained, with which 99% e.e. was obtained.

At the end of the reaction, the enzyme was filtered and washed with a minimum quantity of H_2O-MeOH solution (from 5-10 ml).

The reaction solution was caused to evaporate at reduced pressure and the aqueous phase, suitably basified to pH 8.5, was extracted with ethyl acetate (approximately 70 ml, 4 times) in the presence of NaCl (approximately 5g).

The extraction was monitored by means of HPLC according to the following analysis conditions with a Shimadzu HPLC column: chiral AGP 10 cm x 4 x 5 Ø

Eluent: 2% CH_3CN, V 98% potassium phosphate buffer at 10 mM pH= 4.67

Flow: 0.9 ml/min, UV / visible detection (λ=237 nm)

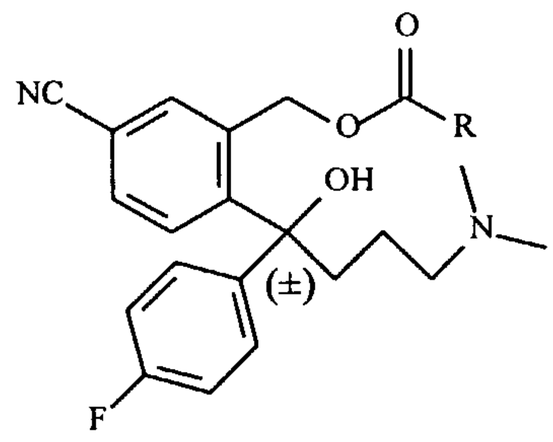
The organic phase containing 4-(4-dimethylamino)-1-(4'-fluorophenyl)-1-(hydroxybutyl)-3-(acyloxymethyl) benzonitrile and part of the diol were evaporated at reduced pressure. The crude reaction product (8 g) is thus obtained and then had to be purified by crystallization.

Example 3: Synthesis of (-)4-(4-dimethylamino)-1-(4'-fluorophenyl)-1-(hydroxybutyl)-3-(hydroxymethyl) benzonitrile

The crude product isolated by the preceding reaction (8 g) was dissolved with diethyl ether (approximately 40 ml), a minimum quantity of ethyl acetate (0.1 ml) was added and the whole was heated gently. Precipitation of a solid was obtained by cooling. The filtrate was subjected to a second crystallization operation and, after cooling to from 0-4°C, there was obtained precipitation of solely the (-)4-(4-dimethylamino)-1-(4'-fluorophenyl)-1-(hydroxybutyl)-3-(acetoxymethyl)-benzonitrile as a white solid (2.2 g) with a purity of 98% and with 99.8% e.e., $[\alpha]_D$= -39.87/-40.00. The solid obtained was subsequently dissolved in 175 ml of 30% NH_3 and in 100 ml of MeOH, the solution was left under agitation for approximately 4 hours and subsequently evaporated to produce 1.9 g of (-)4-(4-dimethylamino)-1-(4'-fluorophenyl)-1-(hydroxybutyl)-3-(hydroxymethyl) benzonitrile .

CLAIMS

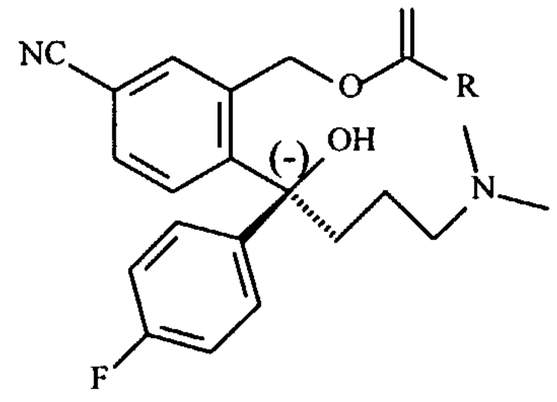
1. A process for preparing escitalopram comprising resolution by means of an esterase from *Aspergillus niger* of a racemic mixture of a compound having formula I



(I)

where R represents a C_1-C_4 alkyl radical, or an aryl radical, in order to provide the corresponding (-) enantiomer having formula II.

(II)



2. A process according to claim 1, characterized in that the resolution is carried out in a solvent constituted by a mixture of an alcohol and water.

3. A process according to claim 2, characterized in that the alcohol is a C_1-C_4 alcohol, preferably MeOH.

4. A process according to claim 2, characterized in that the alcohol and water are present in a proportion of from 0.5-1.5 to 1 by volume, even more preferably at a proportion of 1 to 1.

5. A process according to claims 2 to 4, characterized in that the water is used in the form of a phosphate buffer, preferably a monobasic potassium phosphate buffer.

6. A process according to any one of the preceding claims, characterized in that the resolution is carried out at a temperature of from 15-35° C, preferably

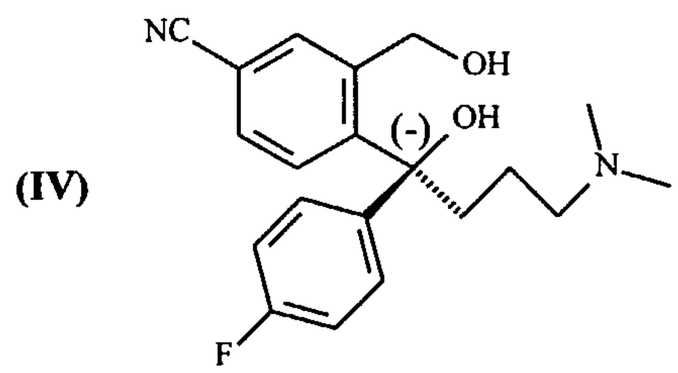
between 20 and 25°C.

7. A process according to any one of the preceding claims, characterized in that the solvent is used at a quantity of from 3-5 litres, preferably from 3.5- 4 litres, per mole of compound having formula I.

8. A process according to any one of the preceding claims, characterized in that the esterase from *Aspergillus niger* is immobilized on resin, preferably epoxy resin.

9. A process according to any one of the preceding claims, characterized in that the esterase from *Aspergillus niger* is used in a quantity of from 2500-3200 units, preferably from 2800-2900 units, per mole of compound having formula I.

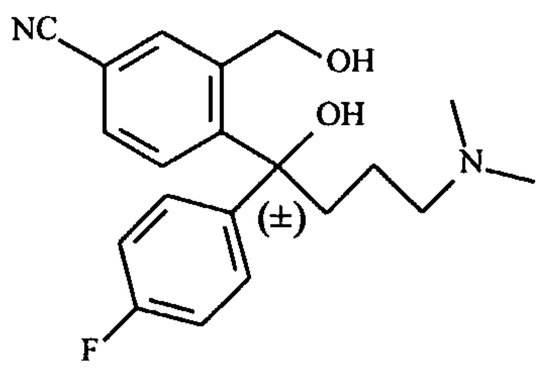
10. A process according to any one of the preceding claims, characterized in that the (-) enantiomer having formula II is converted by means of hydrolysis into benzonitrile having formula IV,



(IV)

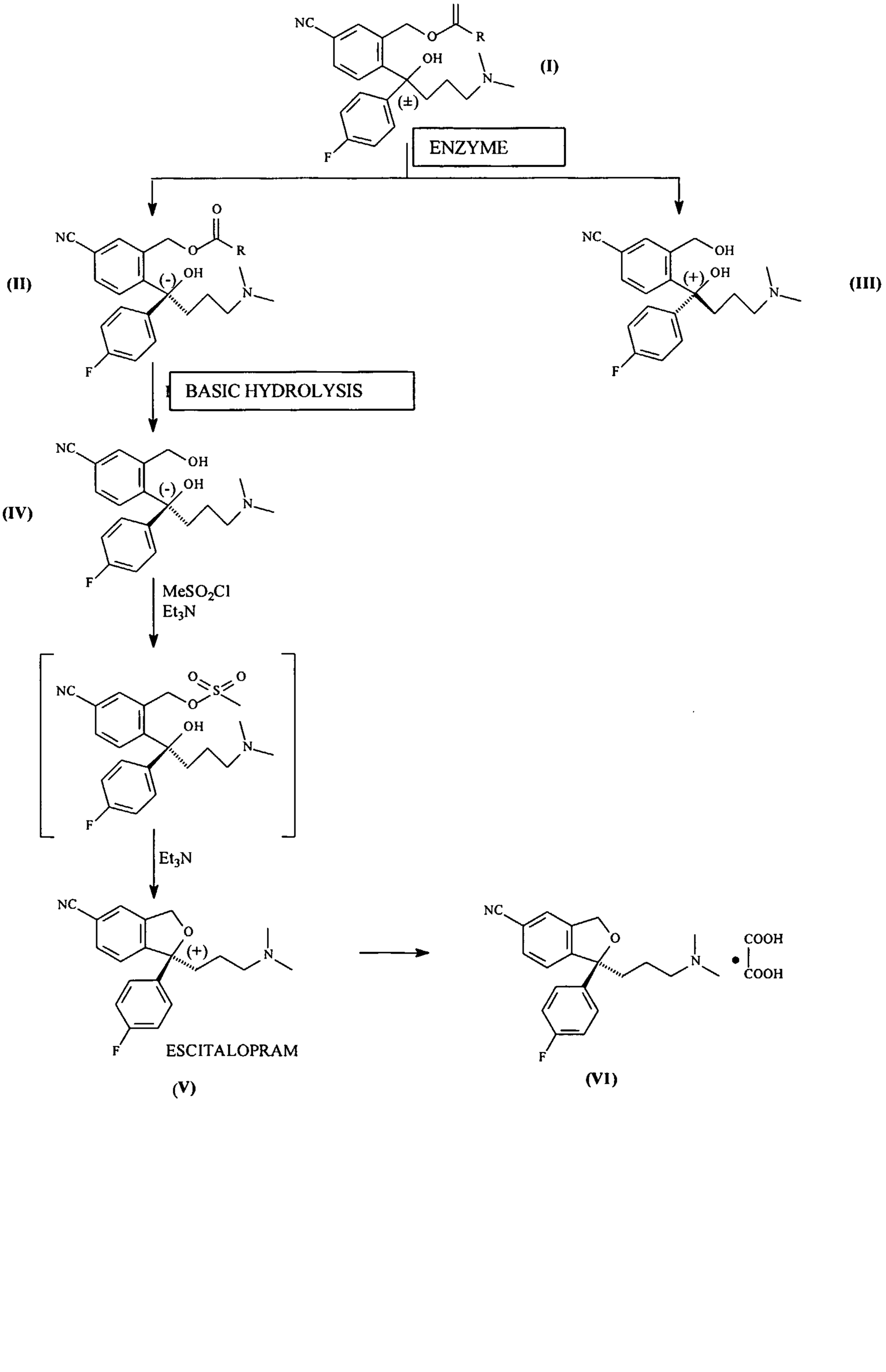
which is subsequently converted into escitalopram by means of condensation of the two hydroxyl groups.

11. A process according to any one of the preceding claims, characterized in that the compound having formula I is obtained by acylation of a compound having formula I'.



(I')

Figure 1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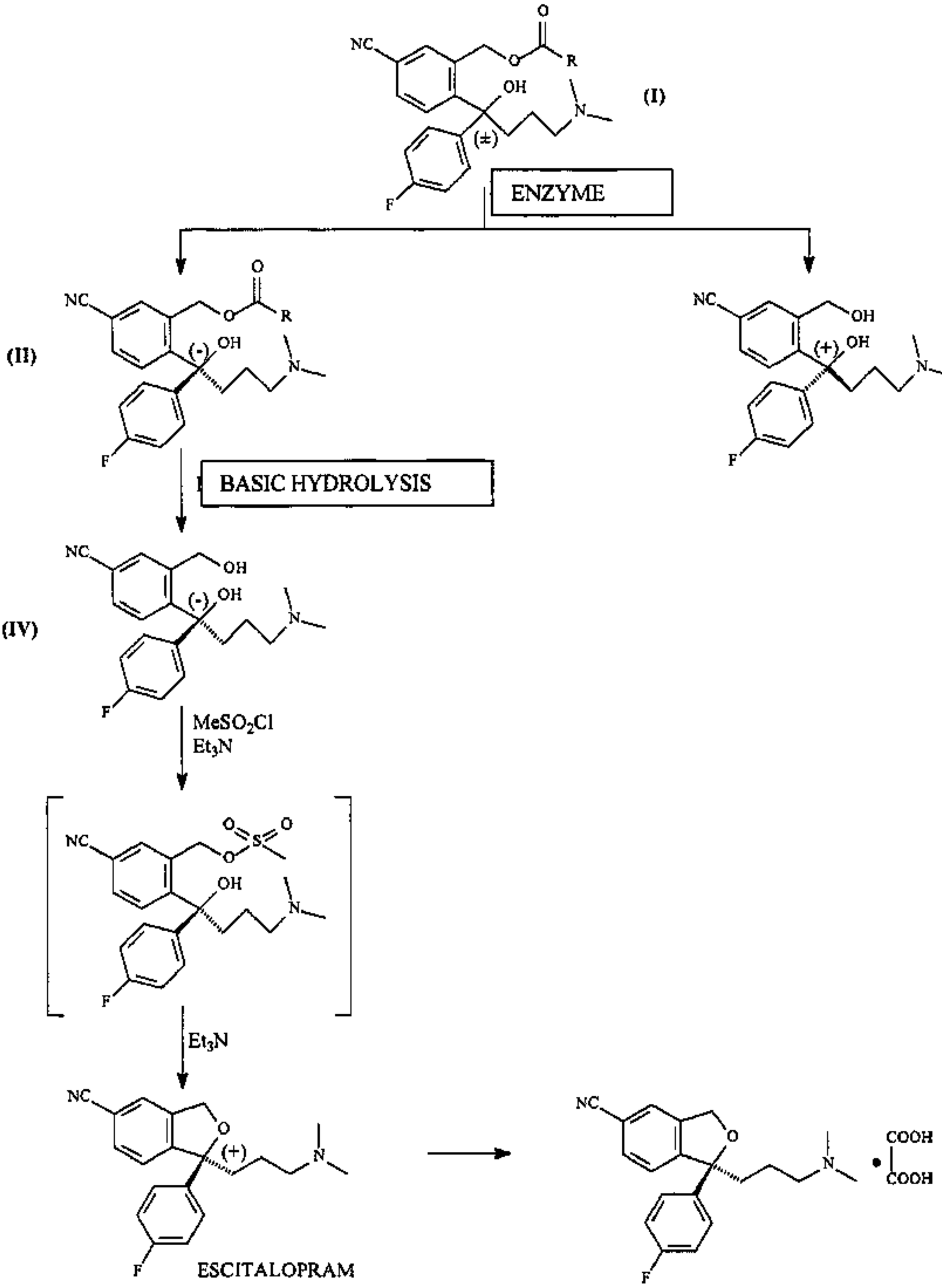
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