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(54) Title: ENZYMATIC PREPARATION OF CHIRAL INDOLE ESTERS

(57) Abstract: The present invention relates to a process for preparing a chiral indole ester by enzymatic resolution using a lipase from *Pseudomonas fluorescens* as the catalyst for the enantioselective hydrolysis of an ester.

WO 2004/104205 A2

TITLE OF THE INVENTION

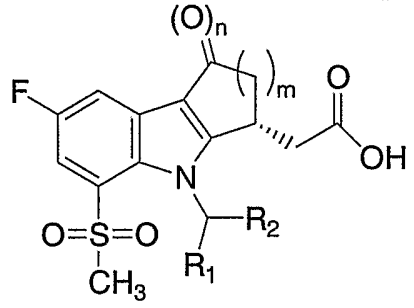
ENZYMATIC PREPARATION OF CHIRAL INDOLE ESTERS

FIELD OF THE INVENTION

The present invention relates to a process for preparing a chiral indole ester by enzymatic resolution using a lipase from *Pseudomonas fluorescens* as the catalyst.

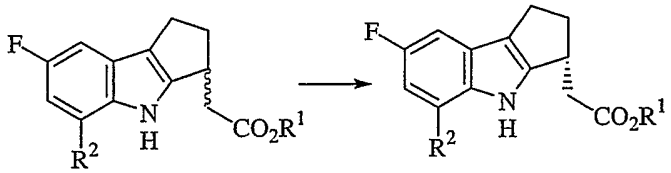
BACKGROUND OF THE INVENTION

Compounds of formula A are antagonists of prostaglandin DP receptor and as such are potential therapeutic agents for the treatment of allergic rhinitis and other disorders mediated through the DP receptor. These compounds are described in PCT Published Application WO03062200.



(A)

In the preparation of compounds of formula (A) it is desired to produce the chiral intermediate I.



Racemate A **I**

wherein R^1 is C_{1-4}alkyl and R^2 is hydrogen or halogen. Chiral compound I may be obtained from a racemic mixture, Racemate A, using conventional chemical processes such as by formation of a salt with an optically active base, followed by separation of the resultant diastereomers, for example by fractional crystallization. However, a chemical resolution process is generally long and tedious, and it would be advantageous if a more convenient method is available for the preparation of chiral compound I, particularly for large-scale applications.

The hydrolysis of esters to acids is a classic chemical manipulation of organic chemistry. However, typical chemical reactions provide no selectivity among possible enantiomeric products when the starting material is racemic. Hydrolases, such as proteases and lipases, have been used and studied

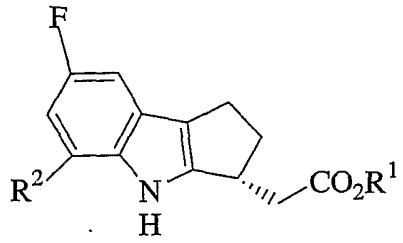
for the asymmetric hydrolysis of organic compounds in both the laboratory and on larger pilot or industrial scales. Because of their stability, abundance and the lack of requirement for expensive co-factors, hydrolases are considered suitable for industrial applications. The use of lipase from *Pseudomonas fluorescens* in asymmetric synthesis is reviewed in Tetrahedron: Asymmetry 2 (8), 733-750, 1991.

SUMMARY OF THE INVENTION

The present invention relates to a process for preparing a chiral indole ester by enzymatic resolution using a lipase from *Pseudomonas fluorescens* as the catalyst.

DETAILED DESCRIPTION OF THE INVENTION

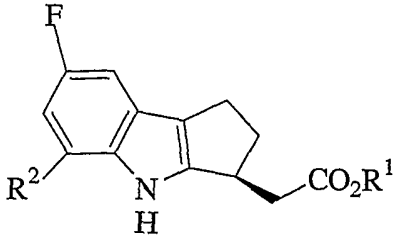
The present invention relates to a process for the preparation of (R)-indole ester of formula I:



I

wherein R^1 is C_{1-4} alkyl and R^2 is selected from hydrogen and halogen, which comprises:

a) hydrolyzing a racemic mixture of indole ester comprising the (R)- indole ester of Formula I and (S)-indole ester of Formula II using an enzymatically effective amount of a Pseudomonas fluorescens lipase to provide a mixture comprising the (R)-indole ester of Formula I and the (S)-indole acid of Formula III; and



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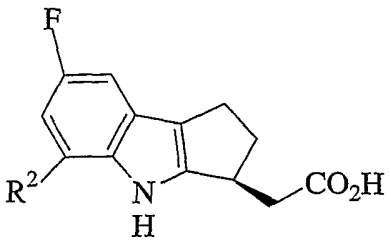


III

b) separating the (R)- indole ester of Formula I from the (S)-indole acid of formula III.

In one embodiment, R^1 is ethyl and R^2 is hydrogen.

In another embodiment, the process further comprises:

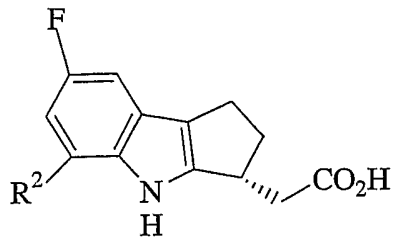
c) reccovering said (S)-indole acid of formula III,

d) esterifying said (S)-indole acid from step c) under racemizing conditions to provide a racemic mixture of indole ester comprising the (R)- indole ester of Formula I and (S)-indole ester of Formula II.

The racemic indole ester, Racemate A, is prepared as described in Reference Example. Enzymatic hydrolysis of Racemate A is carried out using a *Pseudomonas fluorescens* lipase, which may be used as a crude preparation isolated from the producing microorganism, or in a lyophilized powder form, such as Amano AK lipase available from Amano International Enzyme Co., Inc., Troy, Virginia, or the enzyme may be immobilized on solid support, which is also commercially available from Amano. Additionally, whole cell *Pseudomonas fluorescens* may also be used in the process. The lipase is used in an amount sufficient to effectuate the desired transformation; in general the amount of lipase used relative to the substrate is from about 5 to 1 to about 1 to 5 depending on the enzyme specific activity (typically >20000U/g enzyme).

The enzymatic hydrolysis of Racemate A is carried out under conditions that do not impact unduly on the catalytic activity of the lipase, or otherwise interfere with the production of the desired final product. Thus, the reaction may be conducted at a temperature of from about 20 to about 31 °C, preferably at about 24 to about 28°C, and at a pH range of about 6.8 to about 8.5, for example from about 7.5 to about 8.2, preferably from about 7.8 to about 8.2. The enzymatic reaction is carried out in a medium that facilitates substrate-enzyme contact, usually in a buffer to which is added the substrate dissolved in an organic solvent such as N,N-dimethylformamide (DMF). The reaction mixture is agitated to ensure contact of the substrate with the enzyme. The enzymatic hydrolysis is allowed to proceed for a period sufficient to generate satisfactory quantity of the desired (R)-indole ester (compound I); typically, the time period is from 15 to 40 hours, and 95% e.e. may be achieved after about 24 hours.

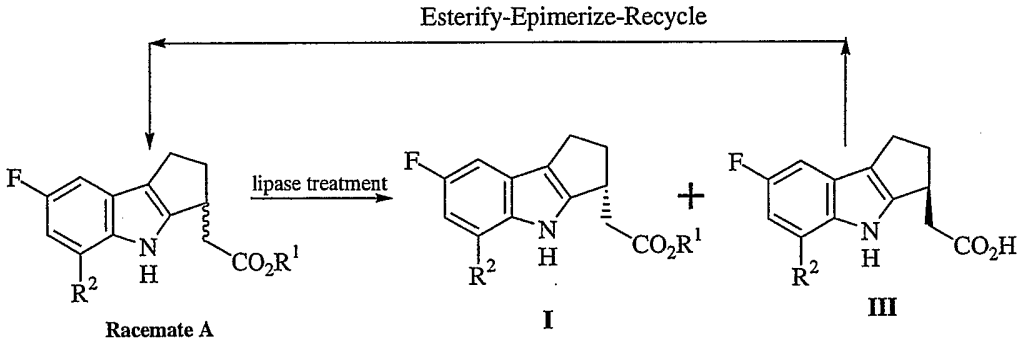
The desired compound I may be separated from the (S)-indole acid of formula III using conventional techniques well known in the art; for example, by treating the enzyme reaction mixture with a base to convert the (S)-indole acid to a salt, followed by partitioning the reaction mixture between water and a suitable organic solvent such as methyl t-butyl ether to remove the salt. The (R)-indole ester (compound I) is hydrolyzed to give the corresponding (R)-indole acid of formula IV, which may be converted to a salt upon treatment with a base; for example, treatment of the acid with dicyclohexylamine provides the dicyclohexylamine salt. The dicyclohexylamine salt formation is carried out in a suitable solvent such as acetonitrile.



IV

Because the enzyme-catalyzed kinetic resolution has only a theoretical yield for the desired (R)-indole ester product of 50%, recycling of the undesired (S)-indole acid is advantageous for industrial-scale synthesis. Therefore, the (S)-indole acid is esterified under racemization conditions to provide Racemate A, which is then cycled through the enzymatic hydrolysis process described above. The (S)-indole acid, compound III, may be recovered as the dicyclohexylamine salt by treating the acid with dicyclohexylamine. The (S)-acid DCHA salt is treated with an alcohol, R^1OH (wherein R^1 is C1-4alkyl), and sulfuric acid at high temperature, for example at reflux, for esterification and epimerization (see Scheme 2). The resulting racemic mixture of the recycled indole ester is then subjected to the enzyme-catalyzed asymmetric hydrolysis under the described conditions.

SCHEME 2



Monitoring the Hydrolysis Reaction

Reaction products were evaluated by the following chromatographic systems along with polarimetric analysis (CD), NMR and mass spectrometry:

HPLC Monitoring System--The time course of the reaction was monitored on a Zorbax C8 reverse-phase column (4.6 mm x 25 cm). The column was developed at ambient temperature with a linear gradient solvent system with a 15-minute run time during which the concentration of solvent B was raised from 50% to 95% [A (2 mM ammonium formate, pH 3.5): B (acetonitrile /solvent A (9:1)] with a flow rate of 1 ml/min.

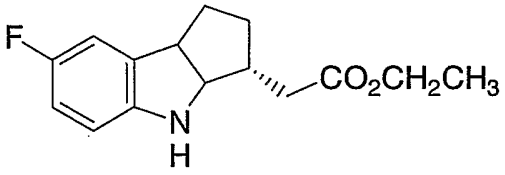
SFC Chiral Column Chromatography--Chirality evaluation of the reaction product was examined on a Chiralcel OJ column running in 15% methanol/carbon dioxide with a flow rate of 1.5 ml/min at 35°C during a 20-minute run time.

LC-Mass--Mass spectrometric analysis was run on a C18 reverse-phase column-equipped mass spectrometer. This column (3.0 x 40 mm) was developed with 2 mM ammonium formate buffer, pH 3.5 with a flow rate of 1 ml/min over 2.8 minutes during which the concentration of solvent B was raised from 5% to 100%.

Below are examples of specific embodiments for carrying out the present invention. The examples are offered for illustrative purposes only, and are not intended to limit the scope of the present invention in any way.

EXAMPLE 1

Preparation of ethyl (3R)-(7-fluoro-1,2,3,4-tetrahydrocyclopenta[b]indol-3-yl)acetate



Method A For the enzyme hydrolysis reaction, 150g of lipase [Amano AK lipase (LAKX09503) from Amano International Enzyme Co. (Troy, Va., USA),] was suspended in 360 ml of 0.1M phosphate bufffer, pH 7.5 in a 2-L three-neck round bottom reaction flask that had been equipped with a mechanical stirrer. To the resulting uniform suspension, 36.5 g crude racemic ethyl (7-fluoro-1,2,3,4-tetrahydro-cyclopenta[b]indol-3-yl)acetate dissolved in 40 ml DMF was added during a 30 minute period. Finally, the dropping funnel was washed with 100ml hexane and the resulting solution was added to the reaction flask. The mixture was then stirred for an additional 30 minutes, and its pH was adjusted to 7.5. After overnight incubation while stirring at room temperature, the pH of the reaction mixture was again adjusted to 7.5 and the mixture was exhaustively extracted with methyl t-butylether (MTBE). The volume of the MTBE extract was reduced to 500 ml by rotorevaporation, and the remaining solution was then washed three times with 10% aqueous sodium bicarbonate and dried over sodium sulfate. The dried MTBE extract was stripped of solvent and the residue was dissolved in a minimum amount of hexane with mild heating, and then cooled to 4°C. After an overnight incubation at 4°C, crystal was recovered from the hexane solution. The recovered crystal was washed with cold hexane and dried. The sample was analyzed by HPLC, LC-Mass and CD after dissolution in the appropriate solvent as previously described. The title compound was confirmed with an enantiomeric excess of 97.9%. The CD of this product showed a negative (-) optical rotation at 275nm when examined in methanol.

Method B To a reaction vessel equipped with a stirrer, temperature control, and pH control (with NaOH (3.8N) addition via a peristaltic pump), add a volume of the mixture from Reference Example to provide 100 g of the racemate, dimethylformamide (2.5 mL/g racemate), and *Pseudomonas fluorescens* Lipase AK-AF (Amano 20, Lot # LAKAF1152102, 840 kU of enzyme/100 g racemate) in buffer (pH 8.0, 0.2M dibasic potassium phosphate in deionized water, sufficient volume to make up 1 liter reaction mixture). The reaction is carried out using the following reactor settings: pH = 8.0, temperature = 28°C, stir rate = 400 RPM. A typical optical purity of 95% ee of the desired ester is obtained at 49% conversion at approximately 24 hours into the reaction. Greater than 99% ee of the desired ester is obtained after 38 hours reaction time.

EXAMPLE 2

Preparation of DCHA salt of (3R)-(7-fluoro-1,2,3,4-tetrahydrocyclopenta[b]indol-3-yl)acetic acid

Once the resolution described in Example 1 (Method B) is complete (e.e ≥98%), ½ volume of acetonitrile is added to the mixture followed by the addition of ½ volume of methyl t-butyl ether (MTBE), and solka-floc (15 wt%). The reaction mixture is stirred at room temperature for ca. 1 hour and filtered. The pad of solka-floc is rinsed with ½ volume of MTBE. The solution is pumped back into the vessel and is further diluted with ½ volume of MTBE. A ½ volume of 4% aq. sodium hydroxide (4 g/L; 0.1 N) is added, and the biphasic mixture is stirred for ca. 15 min, allowed to settle and the layers are separated. The organic layer is then washed twice with ½ volume of a 5 wt% aqueous sodium bicarbonate solution (50 g/L, 2 x 1/2 volume). DMAc (2.5 L/ kg indole ester) is added to the organic layer along with n-heptane (2.5L /kg of indole ester) and *5N* aq. NaOH (0.76L / kg indole ester, 1 equivalent) is added over 5 min at r.t. The biphasic mixture is stirred for 2 hours and allowed to settle. Layers are separated and the organic is washed with water (1.5 L/ kg indole ester). Combined basic aqueous DMAc solution is pumped back into the vessel. MTBE (7.5L /kg of indole ester) is added and the aqueous is neutralized at r.t. to pH~1-2 with 5% aqueous HCl (ca. 0.6 N, 8.5L /kg of indole ester) over stirring and cooling. Layers are separated and the organic is washed twice with water (2 x 3.5L / kg of indole ester). The MTBE solution is filtered (10 μm), concentrated and switched to acetonitrile until KF≤500. The final total volume is adjusted to ca. 6.5L / kg of indole ester. The solution is heated to +50 °C and dicyclohexylamine (DCHA, 0.16 equivalents) is added in one portion and the batch is aged for 1 hour at +50 °C. Remaining DCHA (0.39 equivalents) is added over 1 hour. The mixture is aged at +50 °C for ca. 1 h, allowed to cool to r.t, and further aged for ca. 10 h. The batch is filtered, rinsed with acetonitrile (1 L/ kg of indole ester) and dried in the oven at +40 °C for 24 h.

EXAMPLE 3

Recovery of (S)-(7-fluoro-1,2,3,4-tetrahydrocyclopenta[b]indol-3-yl)acetic acid DCHA salt and lipase enzyme

After extraction of (R)-indole ethyl ester from the reaction mixture in Example 1 (Method A), the pH of the aqueous phase was adjusted to pH 3.5 by slow addition of neat *o*-phosphoric acid. The resulting solution was exhaustively extracted with MTBE. The MTBE extract was then washed with acidified water (pH 3.5), and its volume adjusted to 250ml. Dicyclohexylamine (DCHA) (15.91 mL, 80 mmole) was gradually added to the MTBE extract during a thirty-minute period while the mixture was mechanically stirred. 250ml of additional MTBE was added to the resulting thick paste and stirred for an additional three hours, then filtered. Filtered material was washed with cold MTBE and dried under vacuum. Samples of the dried DCHA salt of the title compound were examined by RP-HPLC, chiral HPLC, LC-MS and CD during the isolation process. The CD data confirmed the expected structure with a (+) optical rotation at about 275nm which is the opposite of the CD of the (R)-indole ethyl ester.

The lipase used in the reaction was recovered by an ethanol precipitation procedure where ethanol was gradually added to the remaining aqueous phase (pH adjusted to 4) after the extractions of the (R)-indole ethyl ester and free (S)-indole acid. The enzyme suspension was cooled down to about 4°C, and the aqueous phase decanted. The precipitated material was washed twice with cold ethanol and then dissolved in water for lyophilization. The lyophilized lipase was then evaluated for catalytic activity.

EXAMPLE 4

Recovery of (S)-(7-fluoro-1,2,3,4-tetrahydrocyclopenta[b]indol-3-yl)acetic acid DCHA salt – Alternate method

Following separation of the organic layer containing the desired (R)-indole ester, the aqueous phase of the biphasic mixture described in Example 2 (1L containing 27.57g of (S)-indole acid) is treated with conc. HCl (ca. 15mL) until pH=3-4. acetonitrile (353mL) and MTBE (786L) are added and the layers are separated. The organic layer is then washed with 190mL of water. The MTBE solution is filtered (10 um), concentrated and switched to acetonitrile (1.25L) until Kf < 5000ug/mL. The final total volume is adjusted to ca. 715mL. The solution is heated to +50 °C and 30% of DCHA (7.8 mL) is added in one portion and the batch is aged for 30 minutes at +50 °C. Remaining DCHA (18.1 mL) is added over 1 hour. The mixture is aged at +50 °C for ca. 1 h, allowed to cool to room temp, and further aged for ca. 10 hr. The batch is filtered, rinsed with acetonitrile (207mL, 7.5mL/g), and dried in the oven at +40 °C for 24 h.

EXAMPLE 5

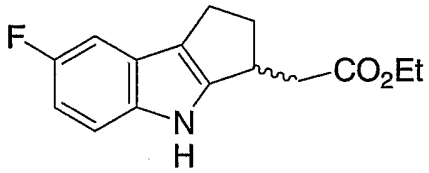
Esterification-racemization to generate ethyl (+/-)-(7-fluoro-1,2,3,4-tetrahydrocyclopenta[b]indol-3-yl)acetate

The DCHA salt of the (S)-indole acid (Example 2, 1.2 g) was esterified and racemized using absolute ethanol (10 ml) and sulfuric acid (0.5 ml) at 95°C for 7 hours. The resulting racemic indole ethyl ester was isolated and then subjected to the enzyme-catalyzed asymmetric hydrolysis under above described conditions in Example 1.

Alternatively, to the DCHA salt add ethanol (10mL/g DCHA salt) and heat to reflux. Add conc. sulfuric acid (5 equiv.) slowly via addition funnel over 1 hour. Age for 18 hours. The mixture is cooled to room temperature and reverse quenched into MTBE (440mL, 100g/L) and 5 wt% K_3PO_4 (66g in 1.32L water), pH=6.0-7.0.

Reference Example

Preparation of (+/-)-(7-fluoro-1,2,3,4-tetrahydrocyclopenta[b]indol-3-yl)acetic acid ethyl ester



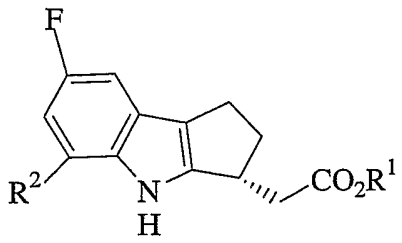
A mixture of ethyl (2-oxocyclopentyl)acetate (1.0 eq.), 2-bromo-4-fluoroaniline (1.05 eq.), and triethylphosphite (1.20 eq.) is treated with 85% phosphoric acid (4 mol%, 0.04 eq.) and the reaction mixture is then warmed to 60 °C under nitrogen. After 7 h the reaction mixture is allowed to cool to room temperature (25-20 °C) and is stirred into a 10/90 volume ratio of triethylamine/ cyclohexane (10 L/Kg of the cyclopentylacetate). Water (5 L/Kg of the cyclopentylacetate) is added to the mixture, and the mixture is stirred for 15 minutes. The layers are separated and the organic phase is washed twice with water (2 x 5 L/kg of cyclopentylacetate), then distilled at constant volume under house vacuum at room temp with a half volume (5 L/kg of cyclopentylacetate) of cyclohexane to remove residual water. Finally, the solvent is switched to dimethylacetamide (DMAC, 1 L/mole cyclopentylacetate) for the cyclization step.

To the above reaction mixture is added triethylamine (2 eq.). Tri-o-tolylphosphine (12 mol%, 0.12 eq.) and palladium acetate (3 mol%, 0.03 eq.) are charged and the solution is degassed with three nitrogen/vacuum purges. The solution is heated at 90 °C for 6 h, then cooled to 20 °C and reverse quenched into a stirred biphasic solution made of a 10 wt% KH_2PO_4 aqueous solution (10 L/kg of cyclopentylacetate) and MTBE (10 L/kg of cyclopentylacetate). The mixture is stirred for 15 minutes and layers are separated. The organic phase is washed twice with water (2 x 5L/kg of cyclopentylacetate).

The organic layer is then filtered through a pad of solka-floc and concentrated under house vacuum at room temp. The solution is then switched to DMF (2.5 L/ kg of cyclopentylacetate) and is used as is for the enzymatic resolution.

WHAT IS CLAIMED IS:

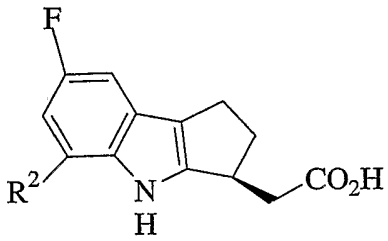
1. A method for preparing a chiral (R)-indole ester of Formula I



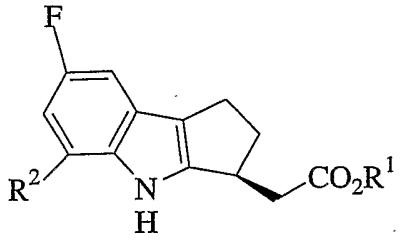
I

wherein R^1 is C_{1-4} alkyl and R^2 is selected from hydrogen and halogen which comprises the steps of:

a) hydrolyzing a racemic mixture of indole ester comprising the (R)-indole ester of Formula I and the (S)-indole ester of Formula II using an enzymatically effective amount of a lipase from *Pseudomonas fluorescens* to result in a mixture comprising the (R)-indole ester of Formula I and the (S)-indole acid of Formula III; and



III



II

b) separating the (R)-indole ester of formula I from the (S)-indole acid of formula III.

2. The method of Claim 1 wherein R^1 is ethyl and R^2 is H.

3. The method according to Claim 2 wherein the hydrolysis is carried out at a pH of from about 6.8 to about 8.5.

4. The method according to Claim 2 wherein the hydrolysis is carried out at a pH of from about 7.8 to about 8.2.

5. The method of Claim 2 wherein said hydrolysis is carried out in a mixture of dimethylformamide and aqueous buffer.

6. The method according to Claim 2 wherein the hydrolysis is carred out in a mixture of dimethylformamide and aqueous buffer, and at a pH of from about 7.5 to about 8.2.

7. The method of Claim 1 which further comprises
recovering the (S)-indole acid of Formula III; and
esterifying said (S)-indole acid with an alcohol R^1OH, wherein R^1 is C_{1-4}alkyl, under racemizing conditions to provide a racemic mixture of indole ester comprising the (R)- indole ester of Formula I and (S)-indole ester of Formula II.

8. The method of Claim 7 wherein said (S)-indole acid of Formula III is treated with ethanol and sulfuric acid to provide said racemic mixture wherein R^1 is ethyl.

9. The method of Claim 7 wherein said (S)-indole acid of Formula III is recovered as the dicyclohexylamine salt.

10. The method of Claim 1 wherein said enzyme is Amano Lipase AK.

11. (3R)-(7-Fluoro-1,2,3,4-tetrahydrocyclopenta[b]indol-3-yl)acetic acid dicyclohexylamine salt.