(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
30 August 2007 (30.08.2007)

PCT

(10) International Publication Number
WO 2007/096314 A2

(51) International Patent Classification: Not classified

(21) International Application Number:
PCT/EP2007/051538

(22) International Filing Date:
18 February 2007 (18.02.2007)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
P-06-28 23 February 2006 (23.02.2006) LV

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 2007/096314 A2

(54) **Title:** ENZYMATIC RESOLUTION OF RACEMIC 3-ARYL-4-AMINOBUTYRIC ACID

(57) **Abstract:** A process for the enzymatic resolution of racemic 3-aryl-4-aminobutyric acid ester into its R-and S-enantiomers, wherein aryl is represented by phenyl group (Phenybut) or p-chlorophenyl group (Baclofen) and ester group by saturated or unsaturated alkyl containing from 2 to 8 carbon atoms. The disclosed process includes the following steps: (1) selective cyclization of 3(S)-aryl-4-aminobutyric acid ester into 4(S)-aryl-2-pyrrolidinone using racemic 3-aryl-4-aminobutyric acid ester in water solution in the presence of -chymotrypsin; (2) acidification of reaction mixture to pH<2.0 and separation of 4(S)-aryl-2-pyrrolidinone and 3(R)-aryl-4-aminobutyric acid ester by extraction; (3) isolation of 4(S)-aryl-2-pyrrolidinone from organic phase and 3(R)-aryl-4-aminobutyric acid ester from water phase and their conversion into respectively R-and S-isomers of 3-aryl-4-aminobutyric acid by acidic hydrolysis.

Enzymatic resolution of racemic 3-aryl-4-aminobutyric acid

Background of the invention

The invention relates to the isolation of pure *R*- and *S*-enantiomers from racemic 3-aryl-4-aminobutyric acids by the means of selective enzymatic resolution. For example pure *R*- and *S*-enantiomers of racemic 4-amino-3-phenylbutyric acid (Phenibut) or 4-amino-3-*p*-chlorophenylbutyric acid (Baclofen) can be obtained by this method. It is known that only *R*-enantiomer of Phenibut is acting as mood enhancer and tranquilizer (Allan et al., *Tetrahedron,* 1990, **46,** No7, 2511-2524). Similarly only *R*-enantiomer of Baclofen is the bearer of antispastic activity (D.R.Hill, N.G.Bowery, Nature, 1981, 290, 149-152). Optical antipodes of these products: *S*-enantiomers of Phenibut and Baclofen are not only less active but even antagonistic to their *R*-antipodes and their presence in the racemic mixture makes necessary to administer higher doses of these drugs.

That is why the elaboration or effective process for the optical resolution of racemic 3-aryl-4-aminobutyric acids opens way to the improvement of the target therapeutic activity of Phenibut and Baclofen.

Description of the prior art

Several methods for the resolution of racemic 3-aryl-4-aminobutyric acids into *R*- and *S*-enantiomers thereof are documented in literature. These are mainly chromatographic separations which include tedious steps of protection and subsequent deprotection of appropriate amino acid (I.Basova et al., *SU* 1432051 (1986). N.Langlois et al., *Tetrahedron,* 1996, **52**, No 48, 15117-15126. R.D.Allan et al., *Tetrahedron,* 1990, **46,** No7, 2511-2524. R.E.Zeile, *Synthesis,* 1991, 1023). Direct resolution of racemic 3-aryl-4-aminobutyric acid was achieved by using columns packed with expensive chiral stationary phase (C.Vaccher, *J. Chromatogr.* 1991, **542,** 502-507). Some methods are represented by cumbersome preferential crystallization of diastereoisomeric salts using optically active bases cinchonidine or L-(-)-α-methylbenzylamine as the resolution agent (M.Soborcinska et al., *Pol. J. Chem.,* 1979, **53**, 435-446. A.F.Wildervanck, et al., *US* 6051734, 2000).

Optical resolution was achieved also by methods based on the combination of chemical and enzymatic transformations (R.Chenevret, M.Desjardins, *Can. J. Chem.,* 1994, **72**, 2312-2317. R.V.Muralidhar, R.R.Chirumamilla et al., *Med. Fac.*

Landbouww. Univ. Gent, 2001, **66,** Nr 3a, 227-232). The *R*-enantiomer of Baclofen was also isolated from its racemic mixture by selective microbial degradation of its *S*-enantiomer therefore excluding the latter from utilization (W.Levadoux et al., *US* 5483765, 1998).

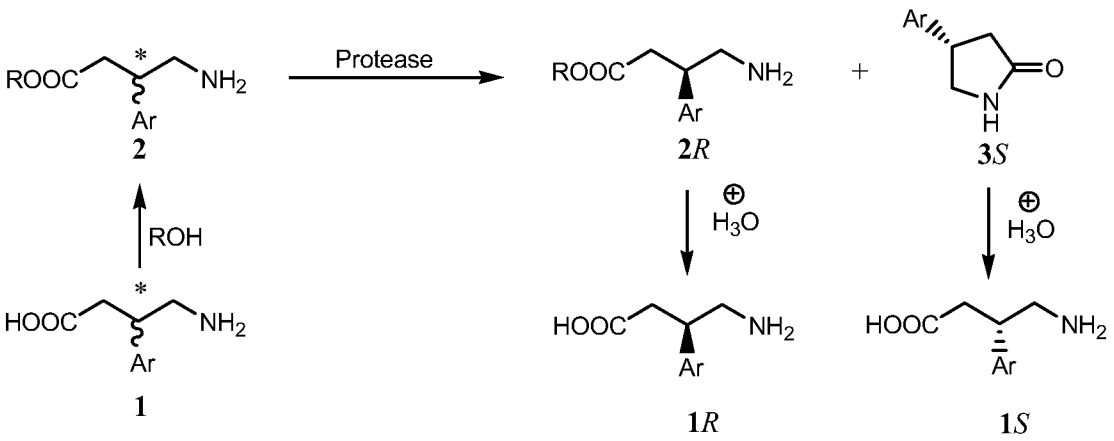
In general, all mentioned methods are not convenient for large-scale production of pure enantiomeric products, because they ignore technological and especially economical aspects of the availability and the cost of reagents and materials. Therefore an easy and effective optical resolution method of racemic 3-aryl-4-aminobutyric acids into their *R*- and *S*-enantiomers was the aim of the present invention.

Summary of the invention

Present invention provides a simple process for inexpensive production of optically pure *R*- and *S*-enantiomers of 3-aryl-4-aminobutyric acid. We have unexpectedly discovered that *R*- and *S*-enantiomers of 3-aryl-4-aminobutyric acid can be produced from readily available racemic ester of 3-aryl-4-aminobutyric acid **2** by its treatment with enzymes selected from protease family which catalyze the hydrolysis of ester group and the formation of peptide bond.

Selective cyclization of 3-aryl-4-aminobutyrate *S*-enantiomer by the action of protease provided the formation of reaction mixture containing only two products: 4(*S*)-aryl-2-pyrolidinone (**3***S*) and 3-aryl-4-aminobutyrate (**2***R*) in *R*-enantiomeric form. We found, that these compounds can be easily separated from each other by conventional extraction technique and then converted into target 3(*R*)-aryl-4-aminobutyric acid (**1***R*) and 3(*S*)-aryl-4-aminobutyric acid (**1***S*) by acidic hydrolysis (Scheme 1).

Scheme 1



Detailed description of the invention

According to the invention, a process for the enzymatic resolution of the racemic mixture of compounds of formula **2** wherein Ar is phenyl or *p*-halo substituted phenyl, R is alkyl group and * marks chiral carbon atom, is provided by the submitting the racemic mixture of formula **2** to the catalyzing action of protease in its free or immobilized state in suitable aqueous or aqueous-organic co-solvent medium. These enzymes react selectively only with *S*-enantiomer of **2** converting it into 4(*S*)-aryl-2-pyrrolidinone (**3***S*), but do not attack the *R*-enantiomer of **2**. We found that the resulting enantiomeric products: 4(*S*)-aryl-2-pyrrolidinone (**3***S*) and 3(*R*)-aryl-4-aminobutyric acid ester (**2***R*), due to their different solubility in two-phase water/organic solvent system at low pH can be effectively separated by extraction and then converted into enantiomeric **1***R* and **1***S* 3-aryl-4-aminobutyric acids respectively by acidic hydrolysis.

The preferred reaction conditions for the enzymatic resolution of racemic esters of 3-aryl-4-aminobutyric acids **2** include:

1. application of α-chymotrypsin in water soluble or in water insoluble immobilized re-usable state for the resolution of 3-aryl-4-aminobutyric acid esters;
2. the preferred substituents R in **2** which are represented by saturated or unsaturated alkyl group containing from 2 to 8 carbon atoms;
3. the usage of aqueous or aqueous-organic co-solvent reaction medium with pH between 6.0-7.0;
4. reaction temperature between 20-40°C;
5. the stirring or shaking of reaction mixture from 1 to 72 hours.

Thus obtained mixture of optically active 4(*S*)-aryl-2-pyrrolidinone (**3***S*) and 3(*R*)-aryl-4-aminobutyric acid ester (**2***R*) can be separated by extraction. Any conventional organic solvents immiscible with water (hydrocarbons such as hexane, benzene, toluene; halogenated hydrocarbons such as chloroform, methylene chloride; esters such as ethyl acetate; ketones, ethers) may be employed for this purpose. Reaction products **2***R* and **3***S* can be isolated from water and organic phases by conventional procedures such as evaporation or crystallization, if necessary. We found that 3(*R*)-aryl-4-aminobutyric acid esters **2***R* are readily soluble in water in

acidic conditions and can be also extracted at neutral pH 7 and isolated from organic phase by evaporation or crystallization, if necessary.

The preferred conditions for the isolation of substantially pure optically active 4(*S*)-aryl-2-pyrrolidinone (**3***S*) from reaction mixture include:

1. the acidification of reaction mixture till pH<2.0;
2. the extraction of 4(*S*)-aryl-2-pyrrolidinone (**3***S*) with organic solvent preferably with toluene, ethyl acetate, benzene or methylene chloride, and the following evaporation of organic phase.

The preferred conditions for the isolation of substantially pure optically active 3(*R*)-aryl-4-aminobutyric acid ester (**2***R*) from reaction mixture, after the elimination of 4(*S*)-aryl-2-pyrrolidinone (**3***S*) by extraction can be performed by two methods including:

1. the evaporation of water phase;
2. the neutralization of water phase till pH 7.0, the extraction of 3(*R*)-aryl-4-aminobutyric acid ester (**2***R*) with organic solvent preferably with ethyl acetate, methylene chloride, toluene or benzene and the evaporation of organic phase.

The following examples are illustrating but not restricting the present invention.

General procedure for the synthesis and enzymatic resolution of racemic 3-aryl-4-aminobutyric acid ester 2

Method 1

A. Thionyl chloride (0.616 ml, 8.4 mM) was added to a cooled to -16°C solution of 3-aryl-4-aminobutyric acid **1** (5.5 mM) in 20 ml of appropriate alcohol. The solution was refluxed for 4 hours, and evaporated under reduced pressure yielding hydrochloric salt of 3-aryl-4-aminobutyric acid ester **2** (98.0%-99.5% purity according to HPLC analysis).

B. Racemic 3-aryl-4-aminobutyric acid ester **2** (25 mg) was dissolved in 5 ml of 0.1 M phosphate buffer (pH 6.0-7.0) with/without addition of 10% of organic co-solvent (dioxane, acetone etc.). Water soluble α-chymotrypsin[1] 5 mg (or 25 mg of α-chymotrypsin immobilized on 1 g of SiO_2 according to the known procedure [K.Watanabe, G.P.Royer, Journal of Molecular Catalysis, 1983, 22, 145]) was added to the reaction mixture and the latter was stirred at 20÷40°C

for 72 hours. The reaction progress leading to the formation of 4(*S*)-aryl-2-pyrrolidinone (**3***S*) was monitored by HPLC.

C. After the end of enzymatic process [2] the reaction mixture was acidified by the addition of 2N HC1 to pH 1.5-2.0 and 4(*S*)-aryl-2-pyrrolidinone (**3***S*) was extracted by 3x20 ml of toluene. Thus obtained organic and water phases were separated and evaporated to dryness giving as residues 4(*S*)-aryl-2-pyrrolidinone (**3***S*) and 3(*R*)-aryl-4-aminobutyric acid ester (**2***R*) respectively which were converted in hydrochloric salts of *S*- and *R*-enantiomers **1***S* and **1***R* of 3-aryl-4-aminobutyric acid by their treatment in boiling 2N HC1 (2 ml) for 20 hours and following evaporation of reaction mixture. Their optical purity was evaluated on the basis of chiral HPLC analysis data[3].

Method 2

The enzymatic resolution of 3-aryl-4-aminobutyric acid (**1**) in steps A and B was realized according to **Method 1**.

C. After the end of enzymatic process [2] the reaction mixture was acidified by the addition of 2N HC1 to pH 1.5-2.0 and 4(*S*)-aryl-2-pyrrolidinone **3***S* was extracted by 3x20 ml of ethyl acetate and evaporated to dryness giving as residue 4(*S*)-aryl-2-pyrrolidinone **3***S*. The water phase was treated with 5N NH_4OH to pH 7 and 3(*R*)-aryl-4-aminobutyric acid ester (**2***R*) was extracted by 3x20 ml of ethyl acetate. Thus obtained organic pahse was evaporated to dryness giving as residue 3(*R*)-aryl-4-aminobutyric acid ester (**2***R*). Both enantiomeric products were converted into hydrochloric salts of *S*- and *R*-enantiomers **1***S* and **1***R* of 3-aryl-4-aminobutyric acid by boiling with 2N HC1 (2 ml) for 20 hours and following evaporation of reaction mixture. Their optical purity was evaluated on the basis of chiral HPLC analysis data[3].

[1] α-Chymotrypsin activity: 65-85 units/mg.

[2] In the case of immobilized α-chymotrypsin the suspension of catalyst after the end of the enzymatic conversion was filtered off, washed by distilled water and re-used repeatedly, if necessary.

[3] Mobile phase - $HClO_4$ (pH 1.0); stationary phase - CROWNPAK CR(+)5a.

The effectiveness of enzymatic resolution and the enantiomeric excess for 3-aryl-4-aminobutyric acids *R*- and *S*-enantiomers obtained by the Methods A and B are presented in Table 1.

Table 1

The conditions of enzymatic resolution process, yields and the enantiomeric excess for 3-aryl-4-aminobutyric acid *R*- and *S*-enantiomers (**1***R* and **1***S*)

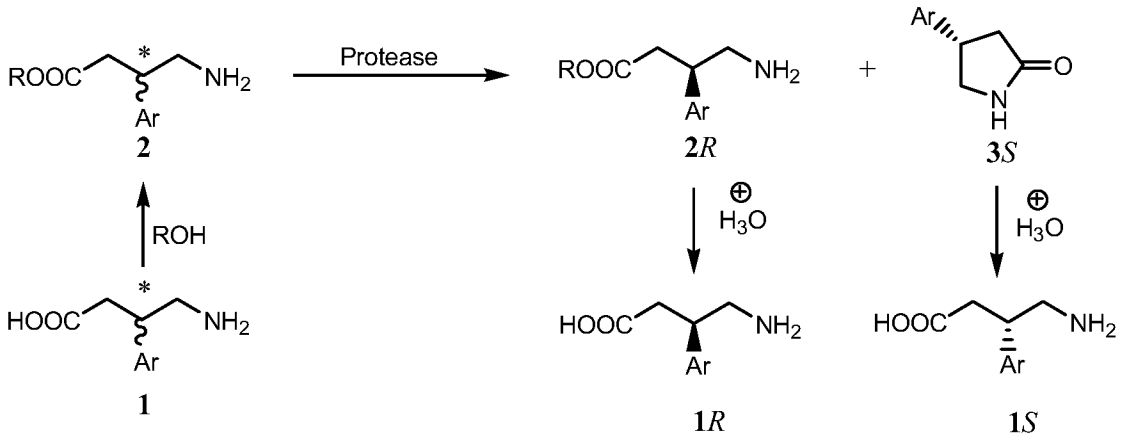
Example (Method)	R	Ar	Time (h)	t (°C)	Medium*	Yield (%) **	Enantiomeric excess (%, *ee*)	
							1*S*	**1***R*
1 (**1**)	Et	Ph	72	20-22	A	66	92	64
2 (**1**)	*i*-Pr	Ph	72	20-22	A	54	76	53
3 (**1**)	*n*-Pr	Ph	72	20-22	A	78	96	76
4 (**1**)	Allyl	Ph	24	20-22	A	68	74	67
5 (**2**)	Allyl	Ph	72	20-22	A	86	96	84
6 (**1**)	*n*-Bu	Ph	24	20-22	A	74	90	72
7 (**2**)	*n*-Bu	Ph	72	20-22	A	98	98	97
8 (**2**)	*n*-Octyl	Ph	72	20-22	A	62	76	60
9 (**1**)	*n*-Bu	*p*-ClPh	72	20-22	B	98	≥98	≥97
10 (**2**)	*n*-Bu	*p*-ClPh	72	20-22	C	98	≥98	≥97

* A - phosphate buffer (pH 6.0-7.0); B - phosphate buffer/dioxane ratio 10:1; C - phosphate buffer/acetone ratio 10:2.

** Reaction yield was calculated on the basis of decrease in the size of 3-aryl-4-aminobutyric acid ester **2** peak on HPLC chart.

We claim:

1. A process of the optical resolution of racemic 3-aryl-4-aminobutyric acid ester (**1**) according to the scheme:



catalyzed by a protease selected from a group consisting of α-chymotrypsin, papain and subtilisin, wherein, Ar is selected from a group consisting of phenyl or *p*-halosubstituted phenyl and R is selected from a group of substituents consisting of saturated or unsaturated alkyl radical containing from 2 to 8 carbon atoms which includes the following steps:

(1) selective catalytic conversion of racemic 3(*S*)-aryl-4-aminobutyric acid ester from racemic 3-aryl-4-aminobutyric acid ester into 4(*S*)-aryl-2-pyrrolidinone (**3***S*);

(2) acidification of reaction mixture to pH<2.0 and separation of 4(*S*)-aryl-2-pyrrolidinone (**3***S*) and 3(*R*)-aryl-4-aminobutyric acid ester (**2***R*) by extraction;

(3) isolation of 4(*S*)-aryl-2-pyrrolidinone (**3***S*) from organic phase and 3(*R*)-aryl-4-aminobutyric acid ester (**2***R*) from water phase by evaporation or crystallization.

2. The process according to claim 1 wherein said protease is α-chymotrypsin.

3. The process according to claim 1 wherein in the step (1) said α-chymotrypsin is used in a water soluble state.

4. The process according to claim 1 wherein in the step (1) said α-chymotrypsin is used in a water insoluble immobilized state.

5. The process according to claim 1 for enantiomeric separation of racemic 3-phenyl-4-aminobutyric acid ester.

6. The process according to claim 1 for enantiomeric separation of racemic 3-*p*-chlorophenyl-4-aminobutyric acid ester.

7. The process according to claim 1 wherein 3-aryl-4-aminobutyric acid ester is selected from a group consisting of saturated and unsaturated C_1-C_8 alkyl esters.

8. The process according to claim 1 for enantiomeric separation of racemic 3-phenyl-4-aminobutyric acid ethyl ester.

9. The process according to claim 1 for enantiomeric separation of racemic 3-phenyl-4-aminobutyric acid *n*-propyl ester.

10. The process according to claim 1 for enantiomeric separation of racemic 3-phenyl-4-aminobutyric acid *i*-propyl ester.

11. The process according to claim 1 for enantiomeric separation of racemic 3-phenyl-4-aminobutyric acid *n*-butyl ester.

12. The process according to claim 1 for enantiomeric separation of racemic 3-phenyl-4-aminobutyric acid allyl ester.

13. The process according to claim 1 for enantiomeric separation of racemic 3-phenyl-4-aminobutyric acid *n*-octyl ester.

14. The process according to claim 1 wherein in step (1) the solvent used is water.

15. The process according to claim 1 wherein in step (1) the solvent used is water-organic co-solvent medium wherein the organic solvent is selected from a group of solvents, consisting of appropriate alkanes, haloalkanes, aralkanes, aromatic solvents, esters, ketones and ethers or mixture thereof.

16. The process according to claim 1 wherein in step (1) the reaction temperature is between 20° and 40°C.

17. The process according to claim 1 wherein in step (1) reaction medium pH is between 6 and 7.

18. The process according to claim 1 wherein in step (3) the water phase is neutralized to pH 7 followed by the extraction of 3(*R*)-aryl-4-aminobutyric acid ester (**2***R*).

19. The process according to claim 1 wherein in steps (2) and (3) an organic solvent is used for extraction, which is selected from a group consisting of hexane, benzene, toluene, chloroform, dichloromethane, dichloroethane, ethyl acetate, methyl acetate, diethyl ether, methyl *t*-butyl ether and mixtures thereof.

20. The process according to claim 1 wherein in steps (2) and (3) hexane is used for extraction.

21. The process according to claim 1 wherein in steps (2) and (3) benzene is used for extraction.

22. The process according to claim 1 wherein in steps (2) and (3) toluene is used for extraction.

23. The process according to claim 1 wherein in steps (2) and (3) chloroform is used for extraction.

24. The process according to claim 1 wherein in steps (2) and (3) dichloromethane is used for extraction.

25. The process according to claim 1 wherein in steps (2) and (3) dichloroethane is used for extraction.

26. The process according to claim 1 wherein in steps (2) and (3) ethyl acetate is used for extraction.

27. The process according to claim 1 wherein in steps (2) and (3) methyl acetate is used for extraction.

28. The process according to claim 1 wherein in steps (2) and (3) diethyl ether is used for extraction.

29. The process according to claim 1 wherein in steps (2) and (3) methyl *t*-butyl ether is used for extraction.

30. The process according to claim 1 wherein in step (3) the isolated 3(*R*)-aryl-4-aminobutyric acid ester (**2***R*) is converted into *R*-enantiomer of 3-aryl-4-aminobutyric acid (**1***R*) by acidic hydrolysis.

31. The process according to claim 1 wherein in step (3) the isolated 4(*S*)-aryl-2-pyrolidinone (**3***S*) is converted into *S*-enantiomer of 3-aryl-4-aminobutyric acid (**1***S*) by acidic hydrolysis.