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Biocatalytic Approach to Enantiomerically Pure β-Amino Acids[1]

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Abstract: β-Aryl-β-amino acids were prepared in good chemical yield and high enantiomeric purity (>95% ee) *via* penicillin acylase-catalyzed hydrolysis of the corresponding *N*-phenylacetyl derivatives. The (*R*)-enantiomers were the fast-reacting isomers in all cases studied. The biocatalytic procedure described employs very simple set of reactions using inexpensive commercially available chemicals and enzyme, and could be easily scaled up.

Introduction.

The recent surge of research activity in the area of β-amino acids is an integral part of more general interest towards non-proteinogenic natural and man-made amino acids which are, apart from their own usually high biological activity,[2] of paramount importance in the synthesis of complex compounds such as peptides with designed conformational properties and biological functions.[3] In particular, most β-amino acids themselves exhibit powerful antibacterial properties and are key constituents of many naturally occurring peptides, terpenes, alkaloids, macrolides and β-lactam antibiotics.[2,4,5] A salient example would be the taxane family of natural and designed diterpenoids containing (2*R*,3*S*)-β-amino-α-hydroxyhydrocynnamic acid crucial for their anticancer activity.[6] Furthermore, the importance of β-amino acids as intermediates for preparing of β-lactams is also well-recognized.[5] In general, β-amino acids are considered as promising biologically active compounds of high pharmaceutical and medicinal interest. In view of potential, mostly biological, applications of β-amino acids their availability in enantiomerically pure forms is necessary.[7] Consequently, in recent years considerable efforts have been expended to devise stereocontrolled methods for their synthesis. It seems that nearly all known methodologies for synthesis of stereochemically defined organic compounds have been applied for the preparation of β-amino acids. Thus, a variety of α- or β-mono-, α,β-disubstituted, carbocyclic and heterocyclic β-amino acids have been prepared in high diastereo- and/or enantiomeric purity using the innate chirality of naturally occurring organic molecules, mostly α-amino acids (manipulation of the chiral pool),[8] stoichiometric,[9] catalytic,[10] and enzymatic (microbial/enzymatic reduction and Michael addition reactions)[11] asymmetric syntheses. On the other hand, in contrast to the prominent role of the biocatalytic approach (enzymatic resolution of racemates) in the production of α-amino acids, its application for the preparation of enantiomerically pure β-amino acids remains, to date, virtually unexplored.[12] Despite the power and flexibility

of synthetic asymmetric approaches, in some cases, especially when simultaneous preparation of both enantiomers is desirable, the biocatalytic approach could be valuable alternative. However, enzymatic resolutions of racemates could be preparatively useful only provided three criteria are met. First, the starting racemate must be cheap and easily prepared. Second, the biocatalytic step should be highly enantioselective and/or enantiomeric purity enhancement of the products should be simple. And finally, there should be a convenient method for the complete separation of the resolved species. Following these criteria we are developing a biocatalytic approach to β-amino acids, which could be in some cases a method of choice, especially from the points of view of generality, economy, and simplicity of experimental procedure. In this paper we would like to report a simple biocatalytic procedure for preparation of enantiomerically pure β-aryl-β-alanines *via* resolution of their racemic *N*-phenylacetyl derivatives by means of penicillin acylase (EC 3.5.1.11) from *Escherichia coli* ATCC 9637.

Results and Discussion

Although the first optimistic results on the enzymatic resolution of β-aminohydrocinnamic acid and β-aminobutanoic acid by α-chymotrypsin[12a] and benzylpenicillinacylase[12b,13] appeared in 1964 and 1977 respectively, β-amino acids still remain challenging targets for biocatalytic resolution techniques. Probably, it is in part because the enzymes, commonly used for the resolution of α-amino acids, such as aminoacylases,[14] amino peptidases[15] or lipases,[16] do not resolve β-amino acids or show low activity and stereoselectivity in this hydrolytic process. Penicillin acylase (PA) (EC 3.5.1.11) from *Escherichia coli* is the unique enzyme that has found industrial application for the production of 6-aminopenicillanic acid, 7-aminodeacetoxycephalosporanic acid, β-lactam intermediate in the synthesis of loracarbef and can also be used for preparation of a variety of semi-synthetic β-lactam antibiotics.[17] Previous investigations of PA substrate specificity had disclosed exceptionally high affinity of this enzyme to the derivatives of phenylacetic acid.[18] Thus, PA is capable of catalyzing hydrolytic cleavage of the phenylacetyl group from amines,[19a] α-[18b,19b,20] and γ-amino carboxylic acids,[21a] α-amino alkylphosphonic acids,[22] peptides[18c,d] sugars[23a] and esters of phenylacetic acid[23] with moderate-to-excellent stereochemical discrimination between corresponding enantiomers. Recently we have demonstrated the successful application of PA for preparation of enantiomerically pure β-per(poly)fluoroalkyl-β-amino acids[24] and all possible stereoisomers of β-amino-α-methyl-β-trifluoromethylbutanoic acid.[25] As a next target, from the view point of generality of PA application for the production of enantiomerically pure β-amino acids, we designed experiment to investigate an ability of this enzyme to resolve β-aryl-β-amino acids, which are of particular interest owing their high biological activity.[26]

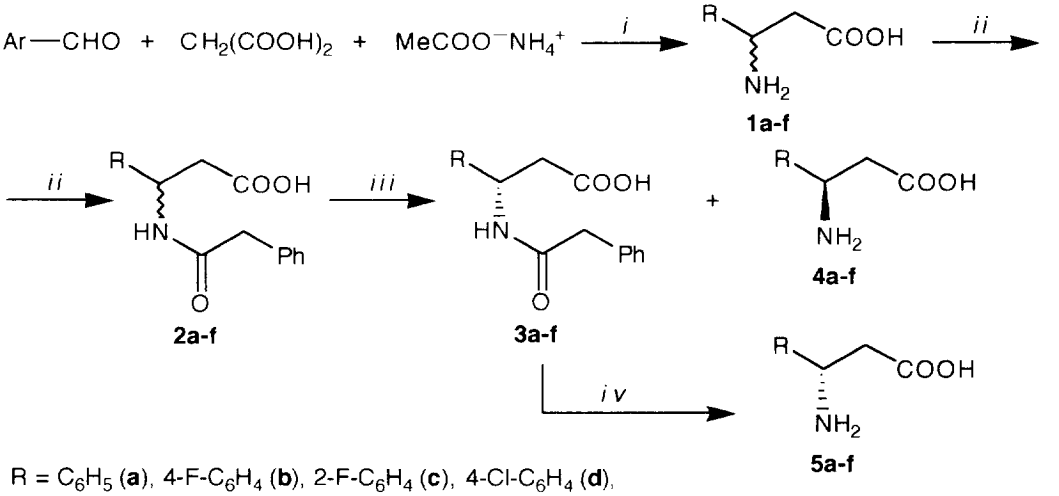
***Synthesis of Racemic β-Aryl-β-Amino Acids* 1a-f.** Looking for the most practical method for preparation of racemic β-aryl-β-amino acids[27] we took note of the little used Rodionow reaction which has, in our opinion, some advantages over more frequently used methods such as addition of ammonia to acrylic acids derivatives or Reformatsky reaction with imines.[28] Rodionow reaction (Scheme 1) consists in interaction between an aromatic aldehyde with malonic acid in the presence of ammonium acetate, which serves at the same time as a base and as a source of amino group.[29] Upon formation, the desired β-amino acid precipitates from solution, which allows the final product **1a-f** to be isolated simply by filtration of the reaction mixture. Without any optimization of the procedure, racemic β-aryl-β-amino acids **1a-f** were easily prepared in 51-74% chemical yields and used for the next phenylacetylation stage.

***Synthesis of Racemic N-Phenylacetyl-β-Aryl-β-Amino Acids* 2a-f.**

Direct phenylacetylation of the amino groups with phenylacetyl chloride under Schotten-Baumann conditions proved to be the most concise and convenient method for the preparation of the corresponding *N*-phenylacetyl derivatives of α- and γ-amino acids.[20-22] Normally, in these syntheses potassium or sodium bicarbonate is used as a base. We have found that application of the standard Schotten-Baumann conditions ($NaHCO_3$) for the phenylacetylation of β-amino acids **1a-f** gives rise to sizable amounts of some by-products which might come from elimination reaction of phenylacetyl amide through α-proton abstraction under the action of such a strong base as sodium bicarbonate. Indeed, the use of triethylamine instead of $NaHCO_3$ allowed us to prepare *N*-phenylacetyl derivatives **2a-f** in good yield by the direct acylation of amino acids **1a-f** with phenylacetyl chloride at low temperature (-5 °C) in water-acetone solutions (Scheme 1). Single recrystallizations of the crude *N*-phenylacetyl derivatives **2a-f** from toluene gave analytically pure samples for biocatalytic resolution.

***Preparative Biocatalytic Resolution of N-Phenylacetyl-β-Aryl-β-Amino Acids* 2a-f.**

Enzymatic resolutions of *N*-phenylacetyl derivatives **2a-f** was accomplished as follows. A 0.1 M aqueous solution of an appropriate **2a-f** was incubated at pH 7.5 at room temperature using 10^{-7} M PA. The course of the enzymatic hydrolytic reaction was monitored by determination of concentration of free amino acid using spectrophotometric *o*-phthalaldehyde method.[30] Upon reaching 50% conversion of starting material the biocatalytic reaction was quenched by acidification with 2 N HCl. It is worth noting, that at the point of 50% conversion the rate of PA-catalyzed hydrolytic reaction slowed down significantly or even stopped, and no sizable influence of the substituent on the aryl ring of the starting *N*-phenylacetyl derivative on the rate of biocatalytic process was observed. The details of the enzymatic resolution procedure are given in the Table 1.



Scheme 1. *Reagents and Conditions*: *i*, MeOH, reflux, 3-6 h; *ii*, water/acetone (1/1), NEt_3, phenylacetyl chloride, –5 °C, 2 hr., then rt, 1 h; *iii*, penicillin acylase, 22-25 °C, pH 7.5; *iv*, 6 N HCl, 50 °C, 11 hr.

Table 1. Biocatalytic Resolution of β-Aryl-β-Amino Acids

Entry	*N*-Phenylacetyl derivative **2a-f**	Quan of **2** (g)	Conc of **2** (mol)	Quan of PA (mL)[a]	Reac time (h)[b]
1	**2a**	1	0.15	1	5
2	**2b**	0.95	0.16	1	5
3	**2c**	1.1	0.16	1	6
4	**2d**	0.9	0.14	0.5	10
5	**2e**	1.7	0.072	1	12
6	**2f**	4	0.076	2	12

[a] Standard solution of 5×10^{-5} M concentration of PA was used. [b] Reaction time for 50% conversion of starting material **2a-b**.

Separation of the resultant amino acids **3a-f** and unconverted *N*-phenylacetyl derivatives **4a-f** was easily achieved with cation-exchange resin using distilled water to isolate **4a-f** and 0.1 N NH_4OH to isolate free amino acids **3a-f**. The enzymatically unconverted *N*-phenylacetyl derivatives **4a-f** were hydrolyzed by 6 N HCl to give amino acids **5a-f**. According to chiral HPLC analysis (see below), both the biocatalytically released, and those obtained by acidic hydrolysis, amino acids **3a-f** and **5a-f** respectively, after a single recristallyzation from water/ethanol were enantiomerically pure (>95% ee). Preliminary experiments on the catalytic constants of the PA-catalyzed hydrolyses of (*R*)- and (*S*)-*N*-phenylacetyl derivatives of β-aryl-β-amino acids have revealed great difference in the rates of (*R*)- and (*S*)-enantiomer hydrolysis. Thus, enantioselectivity of the enzymatic process, expressed as the ratio of the bimolecular rate constants for the hydrolysis of (*R*)- and (*S*)-enantiomers, exceeds 10000.

Determination of Enantiomeric Purity and Absolute Configuration of β-Amino Acids. Ligand-exchange (LE) chiral HPLC analysis is widely used for the determination of the enantiomeric composition and even the preparative separation of α-amino acids.[31] Recently we have shown that this method can be successfully applied also for the quantitative analysis of the enantiomeric purity of β-fluoroalkyl-β-amino acids.[24a,25] After some modification of the previously used conditions we have found that enantiomers of β-aryl-β-amino acids can be separated effectively on the chiral stationary phase containing residues of (*S*)-valine bonded to the surface of a silica matrix (Table 2, experimental part). Under these conditions all racemic amino acids **1a-f** gave two well-separated peaks with integral intensity 50±1. Investigation by this method of the amino acids resolved by PA have shown that all biocatalytically released amino acids **3a-f** have lower retention times then their chemically prepared counterparts **5a-f** (Table 1).

The absolute configurations of amino acids **3a** released by the enzyme and **5a** obtained by chemical hydrolysis of *N*-phenylacetyl derivative **4a** was established as (*R*) and (*S*) respectively by comparison of their $[\alpha]_D$ values with that of described in literature (see experimental). These data and previous results on PA-catalyzed resolution of *N*-phenylacetyl derivatives of α-, β- and γ-amino acids[17-22,24,25] give grounds to believe that all the rest of biocatalytically prepared β-aryl-β-amino acids **3b-f** are the members of the (*L*)-series, and like **3a**, have (*R*)-absolute configuration. Consequently, *N*-phenylacetyl derivatives **4b-f** and amino acids **5b-f** prepared from them by acidic hydrolysis have the (*S*)-absolute configuration. This conclusion is also supported by the similarity in chromatographic behavior of the β-amino acids obtained. Thus, as mentioned above, under

Table 2. Chromatographic Behavior of β-Aryl-β-Amino Acids

Entry	β-amino acid	Retention Times of Enantiomers (min)[a]	
		(*R*)	(*S*)
1	C_6H_5-CH(NH_2)-CH_2COOH	16.5	21.8
2	4-F-C_6H_4-CH(NH_2)-CH_2COOH	21.3	27.5
3	4-Cl-C_6H_4-CH(NH_2)-CH_2COOH	31.3	40.7
4	4-MeO-C_6H_4-CH(NH_2)-CH_2COOH	29.3	43.6
5	3,4,5-(MeO)-C_6H_2-CH(NH_2)-CH_2COOH	28.4	42.3

[a] Chiral stationary phase: column Chiral-Val-Cu = Si 100, 5 μm - (250 x 4.0 mm I.D.), Serva, Heidelberg, Germany. Mobile phase: 5.0 mM $CuSO_4$, flow-rate 0.5-0.75 mL/min, 35 °C; detection at 235 nm.

the conditions of chiral HPLC analysis, retention time of biocatalytically prepared (*R*)-enantiomers is lower than that of (*S*)-enantiomers for all cases studied (Table 2).

Conclusions

We have shown that certain β-aryl-β-amino acids can be obtained in enantiomerically pure form in good yield by PA-catalyzed resolution of their racemic *N*-phenylacetyl derivatives. The method elaborated in this work employs a very simple set of reactions and separations of enzymatically resolved species. The key synthetic step, PA-catalyzed resolution of corresponding *N*-phenylacetyl derivatives was shown to be highly stereoselective giving rise to free (*R*)-β-amino acids in all cases examined. Ready availability of both reagents and enzyme as well as simplicity of experimental procedure would make this method attractive for large scale preparation of enantiomerically pure β-aryl-β-amino acids.

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Experimental

General. ^{1}H-NMR was performed on a Varian VXR-300 (299.94 MHz), Gemini-200 (199.98 MHz) or Bruker WP-200 (188.98 MHz) spectrometer. Tetramethylsilane was used as internal standards in organic solvents and sealed in a glass capillary for D_2O solutions. NMR data are reported in δ units. HPLC analyses were performed on LKB (Sweden) liquid chromatographic system consisting of a model 2150 HPLC pump, a model 7410 injector, a model 2140 detector, a model 2200 recording integrator and model 2155 column oven. Chiral stationary phase: column Chiral-Val-Cu = Si 100, 5 μm - (250 x 4.0 mm I.D.), Serva, Heidelberg, Germany. Mobile phase: 5.0 mM $CuSO_4$, flow-rate 0.5 mL/min, 35 °C; detection at 235 nm. Retention times of enantiomers of β-amino acids are given in Table 1. Optical rotations were measured using a Perkin-Elmer 241 polarimeter. Melting points (mp) are uncorrected and were obtained on a capillary apparatus. Penicillin acylase (EC 3.5.1.11) from *E. coli* ATCC 9637 was prepared and used in soluble form (5×10^{-5} M) as describe earlier.[22] The enzyme concentration was determined as described.[32]

Microanalytical data are given in the Table 3.

Table 3. Microanalytical data

Comp	Calcd			Formula	Found		
	C	H	N		C	H	N
1a	65.43	6.71	8.48	$C_9H_{11}NO_2$	65.41	6.93	8.54
1b	59.01	5.50	7.65	$C_9H_{10}FNO_2$[a]	58.90	5.52	7.51
1c	59.01	5.50	7.65	$C_9H_{10}FNO_2$	59.03	5.41	7.63
1d	54.14	5.05	7.02	$C_9H_{10}ClNO_2$[b]	53.95	4.87	7.17
1e	61.52	6.71	7.17	$C_{10}H_{13}NO_3$	61.71	6.80	7.04
1f	56.46	6.71	5.49	$C_{12}H_{17}NO_5$	56.55	6.91	—
2a	72.06	6.05	4.94	$C_{17}H_{17}NO_3$	72.23	6.21	4.77
2b	67.76	5.35	4.65	$C_{17}H_{16}FNO_3$[c]	67.38	5.79	4.71
2c	67.76	5.35	4.65	$C_{17}H_{16}FNO_3$	67.44	5.49	4.67
2d	64.25	5.08	4.41	$C_{17}H_{16}ClNO_3$	64.23	5.03	—
2e	68.99	6.11	4.47	$C_{18}H_{19}NO_4$	68.99	6.17	4.51
2f	64.33	6.21	3.75	$C_{20}H_{23}NO_6$	64.49	6.42	3.53
3a	65.43	6.71	8.48	$C_9H_{11}NO_2$	65.44	6.73	8.45
3b	59.01	5.50	7.65	$C_9H_{10}FNO_2$	58.97	5.50	7.63
3c	59.01	5.50	7.65	$C_9H_{10}FNO_2$	58.96	5.52	—
3d	54.14	5.05	7.02	$C_9H_{10}ClNO_2$	54.17	5.11	—
3e	61.52	6.71	7.17	$C_{10}H_{13}NO_3$	61.51	6.76	7.18
3f	56.46	6.71	5.49	$C_{12}H_{17}NO_5$	56.47	6.84	5.52
4a	72.06	6.05	4.94	$C_{17}H_{17}NO_3$	72.09	6.11	4.95
4b	67.76	5.35	4.65	$C_{17}H_{16}FNO_3$	67.73	5.32	4.69
4d	64.25	5.08	4.41	$C_{17}H_{16}ClNO_3$	64.25	5.10	4.44
4e	68.99	6.11	4.47	$C_{18}H_{19}NO_4$	69.02	6.14	4.43
5a	65.43	6.71	8.48	$C_9H_{11}NO_2$	65.48	6.77	—
5b	59.01	5.50	7.65	$C_9H_{10}FNO_2$	59.00	5.51	—
5d	54.14	5.05	7.02	$C_9H_{10}ClNO_2$	54.10	4.98	—
5e	61.52	6.71	7.17	$C_{10}H_{13}NO_3$	61.53	6.75	—

[a] Calcd: F, 10.37; Found: F, 10.11. [b] Calcd: Cl, 17.76; Found: Cl, 18.00. [c] Calcd: F, 6.30; Found: F, 6.25.

***Racemic β-Amino Acids* 1a-f.** β-Aryl-β-amino acids **1a-f** were synthesized under the standard conditions of Rodionow reaction starting from appropriate aldehyde, malonic acid and ammonium acetate as described in ref. 30. Crude amino acids **1a-f** were washed with methanol, dried in vacuo and then used for phenylacetylation. β-(Trimethoxyphenyl)-β-alanine because of solubility in methanol was isolated from the reaction mixture by ion-exchange column Dowex-50.

***rac*-β-Amino-β-phenylpropionic acid (1a)**: yield, 53%, 218-219 °C (decomp) [lit.[29d] 220-227 °C, lit.[33] 216 °C, lit.[34] 222 °C]; ^{1}H-NMR (D_2O): ^{1}H-NMR (D_2O): 2.79, 2.88 (ABX, J_{AB} = 18.0 Hz, J_{AX} = 7.5, J_{BX} = 6.7 Hz, 2H, CH_2), 4.45 (dd, J_{HH} = 7.5 Hz, J_{HH} = 6.7 Hz, 1H, CH), 7.18-7.40 (m, 5H, H-arom).

***rac*-β-Amino-β-(4-fluorophenyl)propionic acid (1b)**: 54%, 238 °C; ^{1}H-NMR (D_2O): 2.71, 2.83 (ABX, J_{AB} = 18.0 Hz, J_{AX} = 7.5, J_{BX} = 6.9 Hz, 2H, CH_2), 4.40 (dd, J_{HH} = 7.5 Hz, J_{HH} = 6.9 Hz, 1H, CH), 6.99 (m, 2H, H-arom), 7.26 (m, 2H, H-arom).

***rac*-β-Amino-β-(2-fluorophenyl)propionic acid (1c)**: 64%, 234-236 °C; ^{1}H-NMR (D_2O): 2.73, 2.85 (ABX, J_{AB} = 18.0 Hz, J_{AX} = 7.5, J_{BX} = 6.9 Hz, 2H, CH_2), 4.44 (dd, J_{HH} = 7.5 Hz, J_{HH} = 6.9 Hz, 1H, CH), 7.20 (m, 4H, H-arom).

***rac*-β-Amino-β-(4-chlorophenyl)propionic acid (1d)**: 67%, 237 °C; ^{1}H-NMR (D_2O): 3.03, 3.17 (ABX, J_{AB} = 17.3 Hz, J_{AX} = 7.5, J_{BX} = 6.6 Hz, 2H, CH_2), 4.76 (dd, J_{HH} = 7.5 Hz, J_{HH} = 6.6 Hz, 1H, CH), 7.42 (m, 4H, H-arom).

***rac*-β-Amino-β-(4-methoxyphenyl)propionic acid (1e)**: 61%, 228-229 °C [lit.[29d] 234.5-236 °C, lit.[33] 232 °C, lit.[35] 240-241 °C]; ^{1}H-NMR (D_2O): 2.82, 2.90 (ABX, J_{AB} = 17.1 Hz, J_{AX} = 7.5, J_{BX} = 6.6 Hz, 2H, CH_2), 3.63 (s, 3H, CH_3), 4.70 (dd, J_{HH} = 7.5 Hz, J_{HH} = 6.6 Hz, 1H, CH), 6.85 (m, 2H, H-arom), 7.21 (m, 2H, H-arom).

***rac*-β-Amino-β-(3,4,5-trimethoxyphenyl)propionic acid (1f)**: 74%, 231 °C; ^{1}H-NMR (D_2O): 2.58, 2.70 (ABX, J_{AB} = 16.4 Hz, J_{AX} = 8.2, J_{BX} = 6.3 Hz, 2H, CH_2), 3.58 (s, 3H, CH_3), 3.68 [s, 6H, $(OMe)_2$], 4.41 (dd, J_{HH} = 8.2 Hz, J_{HH} = 6.3 Hz, 1H, CH), 6.61 (s, 2H, H-arom).

***General Procedure for the Synthesis of Racemic N-Phenylacetyl Derivatives* 2a-g.** Phenylacetyl chloride (0.26 mol, 1.3 equiv.) in acetone (30 mL) was added at –5 °C for 0.5 h to the homogeneous solution of racemic β-amino acid (0.20 mol, 1 equiv.) and triethylamine (0.48 mol, 2.4 equiv.) in aqueous acetone (H_2O/MeCOMe as 3/1, 120 mL) under stirring at –5 °C. The mixture was stirred for 2 h at –5 °C and then 3 h at room temperature. The reaction mixture was filtered, acetone evaporated and residue was washed with diethyl ether (3 x 50 mL) to remove unreacted phenylacetyl chloride. The aqueous phase was acidified with 2N HCl up to pH 2.0 and extracted with ethyl acetate (3 x 100 mL). The combined organic layers were dried with $MgSO_4$ and concentrated. The residue was recrystallized from ethyl acetate/hexane to give a crystalline product.

***rac*-β-(*N*-Phenylacetylamino)-β-phenylpropionic acid (2a)**: 69%, mp 134-140 °C; ^{1}H-NMR (CD_3COCD_3): 2.77, 2.89 (ABX, J_{AB} = 15.9 Hz, J_{AX} = 7.5, J_{BX} = 7.2 Hz, 2H, CH_2), 3.50 (s, 2H, $CH_2C_6H_5$), 5.41 (ddd, J_{HH} = 9.0 Hz, J_{HH} = 7.5, J_{HH} = 7.2 Hz, 1H, CH), 7.11-7.50 (m, 10H, H-arom), 7.62 (m, 1H, NH).

***rac*-β-(*N*-Phenylacetylamino)-β-(4-fluorophenyl)propionic acid (2b)**: 71%, mp 159-165 °C; ^{1}H-NMR (CD_3COCD_3): 2.78, 2.87 (ABX, J_{AB} = 15.9 Hz, J_{AX} = 7.5, J_{BX} = 7.2 Hz, 2H, CH_2), 3.52 (s, 2H, $CH_2C_6H_5$), 5.40 (ddd, J_{HH} = 9.0 Hz, J_{HH} = 7.5, J_{HH} = 7.2 Hz, 1H, CH), 7.03-7.40 (m, 9H, H-arom), 7.63 (br.d, J_{HH} = 9.0 Hz, 1H, NH).

***rac*-β-(*N*-Phenylacetylamino)-β-(2-fluorophenyl)propionic acid (2c)**: 65%, mp 143-147 °C; ^{1}H-NMR (CD_3COCD_3): 2.77, 2.89 (ABX, J_{AB} = 16.0 Hz, J_{AX} = 7.5, J_{BX} = 7.2 Hz, 2H, CH_2), 3.55 (s, 2H, $CH_2C_6H_5$), 5.41 (m, 1H, CH), 7.01-7.48 (m, 9H, H-arom), 7.63 (m, 1H, NH).

***rac*-β-(*N*-Phenylacetylamino)-β-(4-chlorophenyl)propionic acid (2d)**:64%, mp 189-193 °C; ^{1}H-NMR (CD_3COCD_3): 2.79, 2.90 (ABX, J_{AB} = 15.9 Hz, J_{AX} = 7.5, J_{BX} = 6.9 Hz, 2H, CH_2), 3.53 (s, 2H, $CH_2C_6H_5$), 5.35 (ddd, J_{HH} = 8.1 Hz, J_{HH} = 7.5, J_{HH} = 6.9 Hz, 1H, CH), 7.01-7.39 (m, 9H, H-arom), 7.87 (br.d, J_{HH} = 8.1 Hz, 1H, NH).

***rac*-β-(*N*-Phenylacetylamino)-β-(4-methoxyphenyl)propionic acid (2e)**: 64%, 155-158 °C; ^{1}H-NMR (CD_3COCD_3): 2.78, 2.91 (ABX, J_{AB} = 15.3 Hz, J_{AX} = 7.1, J_{BX} = 6.9 Hz, 2H, CH_2), 3.51 (s, 2H, $CH_2C_6H_5$), 3.76 (s, 3H, CH_3), 5.35 (ddd, J_{HH} = 8.1 Hz, J_{HH} = 7.5, J_{HH} = 6.9 Hz, 1H, CH), 6.84 (m, 2H, H-arom), 7.11-7.32 (m, 7H, H-arom), 7.63 (br.d, J_{HH} = 8.1 Hz, 1H, NH).

***rac*-β-(*N*-Phenylacetylamino)-β-(3,4,5-trimethoxyphenyl)propionic acid (2f)**: 59%, 157-165 °C; ^{1}H-NMR (CD_3COCD_3): 2.77, 2.84 (ABX, J_{AB} = 15.6 Hz, J_{AX} = 7.5, J_{BX} = 6.9 Hz, 2H, CH_2), 3.53 (AB, J_{AB} = 14.1 Hz, 2H, $CH_2C_6H_5$), 3.67 (s, 3H, CH_3), 3.74 [s, 6H, $(CH_3)_2$], 5.34 (ddd, J_{HH} = 8.7 Hz, J_{HH} = 7.5, J_{HH} = 6.9 Hz, 1H, CH), 7.18-7.36 (m, 7H, H-arom), 7.74 (br.d, J_{HH} = 8.7 Hz, 1H, NH).

***Typical procedure for the enzymatic hydrolysis of rac-N-phenylacetyl derivatives* 2a-f.** Racemic *N*-phenylacetyl derivative (0.011 mol) and KOH (0.012 mol) was dissolved in 150 mL of water and

pH of the solution was adjusted to 7.5 with 1 N KOH. The enzyme solution (mL of 5×10^{-5} M) was added and resulted mixture was stirred at ambient temperature maintaining pH of the solution at 7.4-7.6. The reaction progress was monitored by determination of concentration of amino acid released by the enzyme[30] and at the point of approximately 50% of starting material conversion reaction was quenched with addition of 2 N HCl to make pH 2-3 and extracted with ethyl acetate (3×50 mL). The aqueous phase obtained was heated with activated carbon (65-70 °C, 5 min) and filtered. The clear cold solution was washed with ether (3×50 mL), concentrated *in vacuo*, and passed through cation-exchange resin Dowex-50. Elution by 0.1 N NH_4OH gave ninhydrin-positive (*R*)-amino acid containing fraction which was evaporated to dryness and solid obtained was recrystallized from water or water/ethanol to give chemically and enantiomerically pure free (*R*)-β-aryl-β-amino acid. The ethyl acetate extracts obtained, were combined, dried with $MgSO_4$ and evaporated. Residue was recrystallized from toluene to yield pure (*S*)-*N*-phenylacetyl derivative or heated with 6 N HCl (30 mL) at 50 °C for 11 hr to give free (*S*)-amino acid isolated by Dowex-50 column chromatography [prior chromatography resulted solution was washed with ethyl acetate (3 x 20 mL)].

All biocatalytic resolutions were done in the same way. Yields, mp (decomposition) and $[\alpha]_D$ of (*R*)-**3a-f**, (*S*)-amino acids **5a,b,d,e** and (*S*)-*N*-phenylacetyl derivatives **4a,b,d,e** obtained are listed bellow. ^{1}H-NMR spectra of optically pure products **3-5** are nearly identical to that of racemic samples **1,2**.

(*R*)-β-Amino-β-phenylpropionic acid (3a): yield, 69%, 221-223 °C (decomp), $[\alpha]_D^{20}$ = +6.5 (*c* 0.9 H_2O) [lit.[36] for (*S*)-**3a**, 226-228 °C, $[\alpha]_D^{22}$ = -6.3 (*c* 0.8 H_2O).

(*R*)-β-Amino-β-(4-fluorophenyl)propionic acid (3b): 73%, 220-221 °C, $[\alpha]_D^{22}$ = +3.9 (*c* 0.4 H_2O), $[\alpha]_D^{25}$ = -1.9 (*c* 1 6N HCl).

(*R*)-β-Amino-β-(2-fluorophenyl)propionic acid (3c): 67%, 247 °C, $[\alpha]_D^{22}$ = +3.0 (*c* 0.3 H_2O).

(*R*)-β-Amino-β-(4-chlorophenyl)propionic acid (3d): 70%, 223-225 °C, $[\alpha]_D^{25}$ = -3.33 (*c* 1 6N HCl).

(*R*)-β-Amino-β-(4-methoxyphenyl)propionic acid (3e): 61%, 238-240 °C, $[\alpha]_D^{25}$ = -2.32 (*c* 1 6N HCl).

(*R*)-β-Amino-β-(3,4,5-trimethoxyphenyl)propionic acid (3f): 77%, 217-219 °C, $[\alpha]_D^{25}$ = -6.24 (*c* 1 6N HCl).

(*S*)-β-(*N*-Phenylacetylamino)-β-phenylpropionic acid (4a): 89%, mp 128-132 °C, $[\alpha]_D^{25}$ = -7.0 (*c* 0.5 H_2O).

(*S*)-β-(*N*-Phenylacetylamino)-β-(4-fluorophenyl)propionic acid (4b): 76%, mp 126-130 °C, $[\alpha]_D^{25}$ = -35.61 (*c* 1 CH_3COCH_3).

(*S*)-β-(*N*-Phenylacetylamino)-β-(4-chlorophenyl)propionic acid (4d): 63%, mp 150-154 °C, $[\alpha]_D^{25}$ = -39.24 (*c* 1 CH_3COCH_3).

(*S*)-β-(*N*-Phenylacetylamino)-β-(4-methoxyphenyl)propionic acid (4e): 71%, 122-125 °C, $[\alpha]_D^{25}$ = -47.68 (*c* 1 CH_3COCH_3).

(*S*)-β-Amino-β-phenylpropionic acid (5a): 61%, 222-224 °C, $[\alpha]_D^{20}$ = -6.42 (*c* 1 H_2O) [lit.[36] 226-228 °C, $[\alpha]_D^{22}$ = -6.3 (*c* 0.8 H_2O).

(*S*)-β-Amino-β-(4-fluorophenyl)propionic acid (5b): 69%, 220-222 °C, $[\alpha]_D^{22}$ = -4.0 (*c* 0.5 H_2O), $[\alpha]_D^{25}$ = +1.83 (*c* 1 6N HCl).

(*S*)-β-Amino-β-(4-chlorophenyl)propionic acid (5d): 64%, 220-223 °C, $[\alpha]_D^{25}$ = +3.31 (*c* 1 6N HCl).

(*S*)-β-Amino-β-(4-methoxyphenyl)propionic acid (5e): 59%, 238-239 °C, $[\alpha]_D^{25} = +2.33$ (*c* 1 6N HCl).

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