Biomimetic Transamination of α-Alkyl β-Keto Carboxylic Esters. Chemoenzymatic Approach to the Stereochemically Defined α-Alkyl β-Fluoroalkyl β-Amino Acids

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Biomimetic transamination of the commercially available ethyl 2-methyl-3-keto-4,4,4-trifluorobutyrate (**4**) with benzylamine was shown to provide a simple access to the 2-methyl-3-amino-4,4,4-trifluorobutanoic acids, a hitherto unknown biologically relevant β-amino acid. In sharp contrast to the α-unsubstituted β-keto carboxylic esters the transamination of α-methyl β-keto carboxylic ester **4** proceeds under mild reaction conditions, presumably, due to relative instability of the intermediate (*Z*)-enamine **6**. Diastereoselectivity of the process was found to be controlled by the nature of the base-catalyst allowing for a stereodivergent preparation of (2*R**,3*S**)-**8a** and (2*R**,3*R**)-**8b** diastereomers as dominant reaction products. Preparation of all four diastereo- and enantiomerically pure optical isomers of the 2-methyl-3-amino-4,4,4-trifluorobutanoic acid was effectively accomplished by penicillin acylase-catalyzed resolution of the corresponding diastereomerically pure *N*-phenylacetyl derivatives. The whole process, a stereocontrolled chemoenzymatic approach, including the diastereoselective base-catalyzed [1,3]-proton shift reaction and the enantioselective penicillin acylase-catalyzed resolution, employs a simple set of reactions, inexpensive reagents, and mild reaction conditions, that would render it methodologically useful for preparing biologically interesting α,β-disubstituted fluorinated β-amino acids.

Introduction

Over recent decade β-amino acids have received a great deal of attention due to a wide range of their potential biomedicinal and synthetic applications.[1] Some β-amino acids are naturally occurring compounds serving as key structural components of antibiotics,[2] peptides,[3] and other bioactive materials.[4] Furthermore, the importance of β-amino acids in the rational design of synthetic β-lactams[5] and peptides,[6] in particular β-peptides,[6f,g] has been also well-recognized. In this context, considering the exciting benefits of the fluorine substitution for hydrogen disclosed for the family of α-amino acids,[7] the development of synthetic methodology for preparing fluorine-containing and enantiomerically pure β-amino acids is of particular interest.

As an extension of our studies on the synthesis of fluorine-containing amino compounds of biomedicinal importance,[8] here we report[9] a chemoenzymatic approach to the stereochemically defined novel amino acid α-methyl-β-(trifluoromethyl)-β-alanine, a fluorinated analogue

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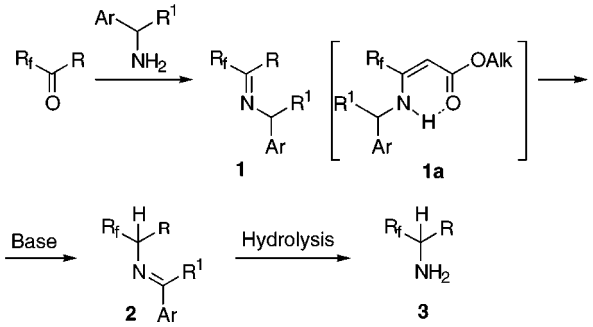
of the amino acid critically involved in the design of carbapenem antibiotics and other biologically relevant compounds.[1,10]

Key stages of our chemoenzymatic approach involve diastereoselective synthesis of the targeted amino acids and their biocatalytic resolution to the enantiomerically pure compounds. The first stage, a methodologically new diastereoselective biomimetic transamination of the fluorinated β-keto carboxylic ester with benzylamine was shown to proceed under mild reaction conditions affording the corresponding Schiff base of the targeted amino acid of either (2*R**,3*S**) or (2*R**,3*R**) stereochemistry, depending on the base-catalyst employed. The second stage, a biocatalytic resolution of the diastereomerically pure amino acids to the pairs of enantiomers was efficiently accomplished via penicillin acylase-catalyzed highly enantioselective hydrolysis of the corresponding *N*-phenylacetyl derivatives. The whole process providing access to all four optical isomers [(2*S*,3*S*), (2*S*,3*R*), (2*R*,3*S*), (2*R*,3*R*)] of the hitherto unknown α-methyl-β-(trifluoromethyl)-β-alanine would be methodologically useful for the development of more general approaches to this class of biologically interesting fluorinated amino acids.

Results and Discussion

Recently we have developed biomimetic, reducing agent-free, reductive amination methodology, referred to as [1,3]-Proton Shift Reaction (PSR),[11,12] for preparing various fluorine-containing amino compounds of biomedicinal and synthetic importance (Scheme 1).[13] A key reaction stage of the method is the base-catalyzed [1,3]-proton shift within 1,3-azaallylic system of imines **1**, **2**, an azomethine−azomethine isomerization, which provides an intramolecular reduction−oxidation process via biomimetic[14] transposition of the imine functionality. Desired amino compounds **3** can be easily released from Schiff bases **2** by an acidic hydrolysis under mild reaction conditions. Synthetic potential of the method has been demonstrated with the efficient preparation of various fluoroalkyl-, fluoroarylamines,[11] α- and β-amino acids[12] **3** (R = Alk, Ar, COOH, CH_2COOH, respectively) starting with appropriate carbonyl compounds and benzylamine, picolylamines, or enantiopure α-phenylethylamines (asymmetric PSR).[11g,12g] However, the azomethine−azomethine isomerizations of imines containing a stereogenic center in the α-position to the imine carbon atom have not been studied so far. Accordingly, apart from the synthetic targets, the present study gains also an additional impetus when considering methodologically new stereo-

Scheme 1



R_f = CF_3, Perfluoroalkyl, CHF_2, $(CF_2)_nH$; R = H, Ph, Bn, *n*-Alk, COOAlk(H), $CH_2COOAlk(H)$; Ar = Ph, C_6H_4N; R^1 = H, Me

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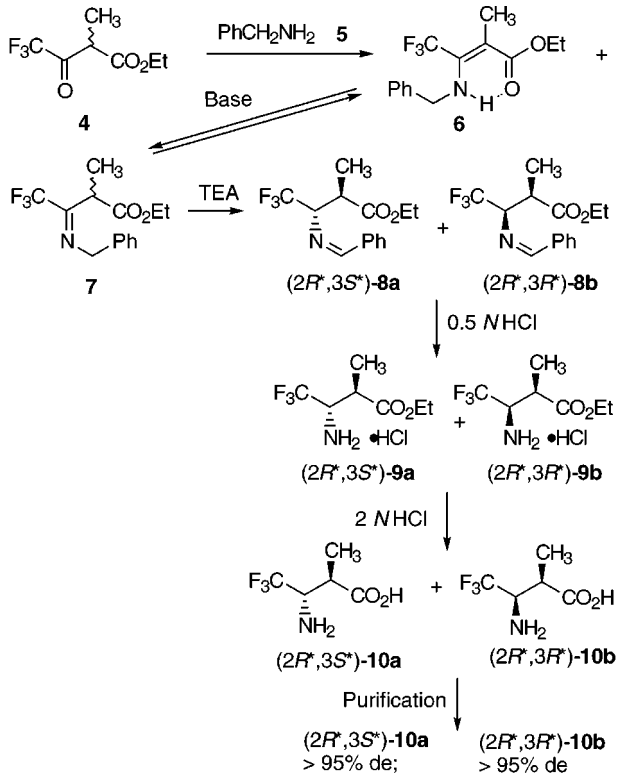
Scheme 2



chemical aspects of the [1,3]-proton-transfer involved in the diastereomeric relations with a neighboring carbon stereogenic center.

Previously we reported that the condensation between the methyl 3-keto-4,4,4-trifluorobutyrate and benzylamine affords, as a main reaction product,[15] the (*Z*)-enamine **1a** (Scheme 1, Alk = CH_3, $R_f = CF_3$, R^1 = H), stabilized by the intramolecular hydrogen bond.[12e] The exclusive formation of the corresponding (*Z*)-enamines was observed also in the reactions of the ethyl 3-keto-4,4,4-trifluorobutyrate with picolylamines.[11e] In sharp contrast to these data, the reaction of the ethyl 2-methyl-3-keto-4,4,4-trifluorobutyrate (**4**) with benzylamine (Scheme 2) gave a mixture of the expected (*Z*)-enamine **6** with ketimine **7** and aldimines **8a**, **8b** in a ratio 1.63/5.76/1.00/1.12, respectively. To account for the result of this condensation one could suggest that the α-methyl group in **6** sterically unfavorably interacts with the trifluoromethyl, overwhelming the stabilizing effect of the intramolecular hydrogen bond in **6**. The unfavorable steric interaction between the methyl and trifluoromethyl groups could be substantially minimized in a more flexible ketimine structure **7**, that renders compound **7** more thermodynamically favorable relative to enamine **6**. The close proximity between the methyl and trifluoromethyl groups in **6** is evidenced from its NMR spectra in which these groups appear as quartets with a quite large through-space coupling constant of 3.3 Hz [^{1}H NMR δ 1.94 (q, J = 3.3 Hz), ^{19}F NMR δ −59.56 (q, J = 3.3 Hz)]. Due to the substantial difference in the chromatographic behavior between enamine **6** and the rest of the reaction products **7**, **8a**, and **8b**, compound **6** was isolated in chemically pure state and fully characterized. Enamine **6** is relatively stable in a toluene solution at room temperature, while an addition to the solution catalytic amounts of a base (NEt_3, $BnNH_2$) causes the corresponding enamine−azomethine isomerization to give, after complete equilibration, a mixture of **6** with **7** in a ratio similar to that observed in the reaction of keto-ester **4** with benzylamine (*vide versa*).

As we have shown earlier, the isomerization of α-unsubstituted enamines of type **1a** (Scheme 1) requires rather forced reaction condition, such as prolong refluxing in a solution of triethylamine (TEA).[12f] Therefore, the formation of the isomerized products **8a** and **8b** under the conditions of keto-ester **4** condensation with benzylamine was quite surprising. Assuming that the formation of Schiff bases **8a** and **8b** might suggest facile benzylamine-catalyzed isomerization of enamine **6** to ketimine **7** and further to **8a** and **8b**, we performed two additional experiments to explore the catalytic activity of the amine toward compound **6** and its α-unsubstituted analogue **1a**. Thus, each enamine **1a** and **6** was dissolved in neat benzylamine at room temperature and the course of the reaction was monitored by ^{19}F NMR. The results of these experiments (24 h at room temperature) were totally different; while enamine **6** was completely isomerized to give a mixture of aldimines **8a** and **8b** in 86% yield, compound **1a** was isolated (96% yield) chemically intact. These data suggest that for the transformation of enamines of type **1a**, **6** to the targeted Schiff base of β-amino acids, the rate-determining step is the enamine−azomethine isomerization, whereas the rate of the following azomethine−azomethine transformation of the corresponding ketimines to the final products is comparable with that of the isomerizations of *N*-benzyl ketimines derived from alkyl(aryl) trifluoromethyl ketones.[11d]

Due to the strong steric influence of the trifluoromethyl group[16] on the neighboring substituents in the products **8a** and **8b**, relative configuration of the diastereomers could be deduced from their ^{1}H NMR spectra. Thus, considering the Newman projections of thermodynamically favorable conformers of (2*R**,3*S**) and (2*R**,3*R**) diastereomers (Figure 1) one can presume a steric impact of the trifluoromethyl group on the methyl in the former, and on the ethoxycarbonyl group in the latter diastereomer. Indeed, in the ^{1}H NMR spectrum of (2*R**,3*S**)-**8a** the methyl group appears as a doublet of quartets with a large through-space HF-coupling constant [δ 1.39 (dq, J_{HH} = 7.2 Hz, J_{HF} =1.5 Hz)], while the effect of the trifluoromethyl group on the methyl in (2*R**,3*R**)-**8b** diastereomer is virtually undetectable [δ 1.24 (dq, 3 H, J = 7.2 Hz, 0.6 Hz)]. On the other hand, the ethoxycar-

(15) In this condensation, apart from the (*Z*)-enamine **1a** the corresponding *N*-benzyl-3-(*N*-benzylamino)-4,4,4-trifluorocrotonamide is also obtained as a minor reaction product.

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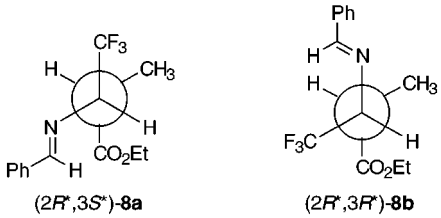

Figure 1. The Newman projections of (2*R**,3*S**)-**8a** and (2*R**,3*R**)-**8b** diastereomers.

bonyl group in the ^{1}H NMR spectrum of (2*R**,3*S**)-**8a** appears as a regular quartet [δ 4.04 (q, 2 H, J = 7.2 Hz)], whereas the CO_2Et group in the ^{1}H NMR spectrum of (2*R**,3*R**)-**8b** is observed as an AB-system [δ 4.14, 4.20 (AB, 2 H, J = 7.2 Hz, J_{AB} = 10.8 Hz)], implying nonequivalence of the methylene protons due to the close proximity with the trifluoromethyl group. The final determination of the relative configuration of diastereomers (2*R**,3*S**)-**8a** and (2*R**,3*R**)-**8b** was accomplished by single-crystal X-ray analysis of the diastereomerically pure free amino acid (2*R**,3*S**)-**10a** (*vide infra*), which confirmed the stereochemical assignments made on the basis of the ^{1}H NMR spectra.

For preparative isomerization of the mixture **6**/**7** to aldimines **8a** and **8b** we applied our standard procedure[11d] using TEA as a base, which could be easily removed in *vacuo* after completion of the process. We have found that the isomerization smoothly occurs at room temperature (22−26 °C), albeit with a moderate reaction rate. Thus, after 48 h the ratio **8a**/**8b**/**6**/**7** was 5/3/1/1, respectively (^{19}F NMR). At an elevated temperature (40−45 °C) the isomerization proceeded with a higher reaction rate allowing for complete and clean transformation of the mixture **6**/**7** to aldimines **8a** and **8b** within a convenient time span (24−18 h, 89% yield). The ratio of thus obtained diastereomeric Schiff bases **8a** and **8b** was found to be 1.7/1.0 (^{1}H and ^{19}F NMR) that is different and opposite in a sense of diastereomeric preferences to the ratio (**8a**/**8b** 1.00/1.12) obtained directly in the condensation of keto-ester **4** with benzylamine (*vide versa*). Assuming that the stereochemical outcome of the isomerization could be a function of the nature of base used and/or reaction conditions applied, we designed a series of additional experiments to get insight in to the nature of the diastereoselectivity of the isomerization. Since ketimine **7**, obtained by the condensation of keto-ester **4** with benzylamine, contained some 20% of the already isomerized products **8a** and **8b**, we investigated the diastereoselectivity of the isomerization using enamine **6**, available in chemically pure form, as starting material. The results obtained are collected in Table 1. As one can see from the table, the rate and diastereoselectivity of the isomerization under study are a function of the nature of the base used. Thus, in solutions of strong bases such as 1,5-diazabicyclo[4.3.0]-5-nonene (DBN) (entry 7) or 1,8-diazabicyclo[5.4.0]-7-undecene (DBU) (entry 8) the isomerization was virtually completed in 1 h, while the 1,4-diazabicyclo[2.2.2]octane (DABCO) (entry 6) and TEA-catalyzed isomerizations (entries 1−3), even at the higher temperatures, necessitated some 10 and 24 h, respectively, for completion of the reactions. Interestingly, the diethylamine was much more effective than TEA in catalyzing the isomerization (entries 4, 5 vs 1, 2), that could be accounted by the difference in the steric properties of these bases.[11e] The

Table 1. Isomerizations of (*Z*)-Enamine 6 to Schiff Bases (2*R,3*S**)-8a, (2*R**,3*R**)-8b[a]**

entry	base	time, h	conversion,[b] %	**8a**	**8b**	yield
1	TEA	24	<80	70.0	30.0	−
2	TEA	40	>98	70.0	30.0	91
3	TEA	24[c]	>98	69.3	30.7	74
4	$NHEt_2$	1	78	67.5	32.5	−
5	$NHEt_2$	24	>98	67.7	32.3	69
6	DABCO	10−24	>98	54.3	45.7	77
7	DBN	1−24	>98	32.7	67.3	77
8	DBU	1−24	>98	30.6	69.4	79

[a] All reactions were run under oxygen-free argon atmosphere at 23−25 °C (for DABCO-catalyzed reaction at 35 °C) in a solution of the base indicated. Relative configuration of the products (2*R**,3*S**)-**8a** and (2*R**,3*R**)-**8b** was determined by X-ray analysis[9,18] of the diastereomerically pure amino acid **10a** obtained from (2*R**,3*S**)-**8a**, see the text. Ratio of the diastereomers **8a** and **8b** was determined on the crude reaction mixtures by ^{19}F NMR. [b] Determined on the crude reaction mixtures by ^{19}F NMR. [c] At 40 °C.

diastereoselectivity of the isomerizations was not high; however, the opposite sense of the stereochemical preferences depending on the nature of base used was rather surprising. Thus, the TEA and diethylamine-catalyzed isomerizations (entries 1−5) afforded a mixture of products with substantial domination of the (2*R**,3*S**)-diastereomer, while the reactions conducted in solutions of DBN and DBU favored the formation of the opposite (2*R**,3*R**)-diastereomer (entries 7, 8). Most interestingly is that the isomerization catalyzed by DABCO, the basicity of which is higher than that of TEA or diethylamine but lower than that of DBN or DBU, was the least stereoselective, giving rise to some 8.6% de of the (2*R**,3*R**)-diastereomer (entry 6).

Mixtures of Schiff bases **8a** and **8b**, obtained in the TEA- and DBU-catalyzed reactions and thus containing as a dominant diastereomer (2*R**,3*S**)-**8a** or (2*R**,3*R**)-**8b**, respectively, were hydrolyzed first under mild reaction conditions to deprotect selectively amino function giving rise to amino-esters hydrochlorides **9a** and **9b** (Scheme 2), and then, in the presence of 2 N HCl to afford free amino acids **10a** and **10b**. Direct hydrolysis of Schiff bases **8a** and **8b** to amino acids **10a** and **10b**, without isolation of the intermediate amino-esters **9a** and **9b**, gave lower yield of the targeted amino acids. Mixtures of the amino acids, containing some 40% de of (2*R**,3*S**)-**10a** or (2*R**,3*R**)-**10b** diastereomer, respectively, were recrystallized each from acetone/ether solutions to afford amino acids (2*R**,3*S**)-**10a** and (2*R**,3*R**)-**10b** of >95% diastereomeric purity, as determined by chiral HPLC analysis.[17] Relative configuration of the diastereomerically pure (2*R**,3*S**)-**10a** was established by X-ray analysis (*vide versa*).[18]

Biocatalytic resolution of α-amino acids is a well-tried, extensively used approach for preparing enantiomerically

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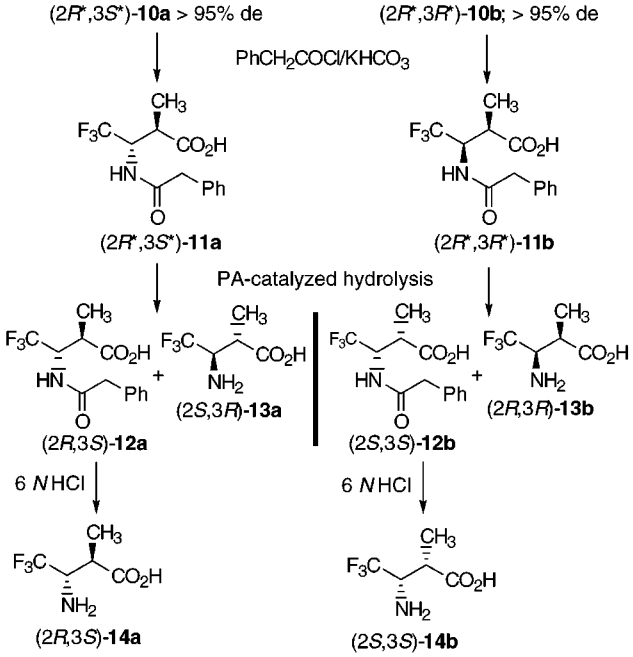
(18) Crystals of amino acid **10a** were grown from water−ethanol solution. Crystal data for **10a**: $C_5H_8F_3NO_2$, space group PBCN (no. 60). Unit cell: a = 12.640(3) Å, b = 15.542(5) Å, c = 9.462(8) Å, V = 1858(2) Å^3. Diffraction data were measured on an Enraf-Nonius CAD4 diffractometer (Mo radiation). 1063 Unique reflections were considered and used in the analysis. The structure was solved by Patterson method. The final R factor was 0.039. Complete crystallographic data were deposited with the Cambridge Crystallographic Data Centre, at the time of preliminary communication publication,[9] and are available, on request, from the Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 1EZ, UK.

pure compounds from the corresponding racemic derivatives.[19] By contrast, biotransformations of β-amino acids, which would allow for preferential formation of a single enantiomer, remain, to date, virtually unstudied.[8c] The challenge associated with a biocatalytic resolution of β-amino acids is largely due to the fact that the enzymes, normally used for the resolution of α-amino acids, such as, for instance, aminoacylases[20] and aminopeptidases,[19c] do not resolve β-amino acids. A fortunate exception among the amidases suitable for resolution of nonconventional amino acids is the penicillin acylase from *Escherichia coli* (PA) (EC 3.5.1.11), a unique enzyme most known so far because of its industrial application for modification of natural and synthetic β-lactam compounds.[21] This enzyme is characterized by the extraordinary effective hydrophobic binding of an acyl group of its substrates in the active center[22] and very high stereospecificity.[22d] Being very specific to a phenylacetyl moiety, as an acyl group, PA at the same time has rather broad specificity to the amino component.[22b,c,d,23] Due to this property, PA has been successfully applied for biocatalytic *N*-phenylacetyl protection[23a] and deprotection of amino groups in peptides, amino acids derivatives,[23a,b] and nucleosides,[23b] enantioselective biocatalytic resolution, via enantioselective hydrolytic cleavage of a phenylacetyl group, of α-amino carboxylic acids,[22c,d,23e] γ-amino carboxylic acids,[23f,g] α-amino alkylphosphonic[23c] and α-aminoalkylphosphonous acids.[23d] In particular, highly enantioselective PA-catalyzed resolution of β-amino acids have been reported recently by our[8c,24] and Cardillo, Tomasini groups.[25] Accordingly, for the biocatalytic resolution of the target amino acids (2*R**,3*S**)-**10a** and (2*R**,3*R**)-**10b** we decided to try the developed by us PA-assisted procedure.[8c,24]

Scheme 3



N-Phenylacetyl derivatives (2*R**,3*S**)-**11a** and (2*R**,3*R**)-**11b** were synthesized with excellent isolated yields via Schotten−Baumann procedure by treatment of water−acetone solutions of β-amino acids **10a** and **10b** in the presence of potassium bicarbonate with phenylacetyl chloride at low temperature (−5 °C) (Scheme 3). For preparative enzymatic resolutions of substrates **11a** and **11b** PA (EC 3.5.1.11) from *E. coli* atcc 9637[22] was used. The biocatalytic reactions were performed in aqueous solution of pH 7.5 at room temperature in the presence of 10^{-6} M PA. The course of the hydrolytic reactions was monitored by consumption of 5% NH_4OH and at an appropriate point, to obtain 50% conversion of the starting material, the process was stopped by adjusting the pH of the solutions to 2 with a 1 M HCl. Substantial differences in the physical properties of the resolved products allowed for complete separation and simple isolation of the nonhydrolyzed *N*-phenylacetyl derivatives **12a**, **12b** and accumulated free amino acids **13a**, **13b**. Thus, compounds **12a**, **12b** were extracted from the reaction solutions with ethyl acetate, while amino acids **13a**, **13b** were isolated using cation-exchange resin Dowex-50. The second pair of optical isomers of α-methyl-β-(trifluoromethyl)-β-alanine **14a** and **14b** were prepared by chemical hydrolysis of enzymatically unconverted *N*-phenylacetyl derivatives **12a** and **12b**. Chiral HPLC analysis[17] of crude amino acids **13a**, **13b** and **14a**, **14b** revealed their >95% optical purity, that suggest excellent enantioselectivity of the biocatalytic reaction. Preliminary kinetic studies have

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shown that the enantioselectivity of the enzymatic resolution process, expressed as the ratio of the second-order rate constants for the hydrolysis of the enantiomers, exceeds 500.

Absolute configuration of the resolved products was assigned on the basis of the following data. As it was shown earlier, PA is extremely stereoselective toward (L)-enantiomers of the corresponding *N*-phenylacetyl derivatives of α-amino carboxylic acids and α-amino alkylphosphonic acids regardless of the nature of an acidic function (*vide versa*).[22d,23c,d] The ratio of second-order rate constants for the PA-catalyzed hydrolysis of L- and D-enantiomers could be as high as 150 000.[22d] The same sense of enantiopreferences was revealed also for the PA-catalyzed hydrolyses of the *N*-phenylacetyl derivatives of β-amino[24,25] and γ-amino carboxylic acids.[23f,g] In particular, the corresponding (*R*)- and (*S*)-enantiomers[26] were shown to be fast-reacting stereoisomers in the PA-catalyzed resolutions of β-aryl β-amino[24d] and β-alkyl β-amino[8c] carboxylic acids, respectively. Furthermore, the resolutions of β-(fluoroalkyl)-β-alanines, in particular *rac*-4,4,4-trifluoro-3-(*N*-phenylacetylamino)butanoic acid, followed the same sense of stereochemical preferences affording a mixture of the corresponding enantiomerically pure (>95% ee) (*R*)-configured amino acid and unreacted *N*-phenylacetyl derivative of (*S*) absolute configuration.[24c] Moreover, recent study by Cardillo and Tomasini[25] into the PA-catalyzed resolutions of (2*R**,3*R**)-2-substituted-3-aminobutanoic acids, in particular α-methyl-β-amino butanoic acid, have shown that the sense of stereodiscrimination in the enzymatic hydrolysis is not influenced by the nature of the α-substituent giving rise to the corresponding (2*S*,3*S*)-amino acids and leaving intact (2*R*,3*R*)-*N*-phenylacetyl derivatives. Finally, an attempt to resolve with the PA the *N*-phenylacetyl derivative of α-methyl-β-alanine, led to almost racemic product, suggesting that the bearing a methyl group α-stereogenic center is not involved in the enantioselective step of the PA-catalyzed hydrolytic reaction. Accordingly, amino acid **13a** and nonhydrolyzed *N*-phenylacetyl derivative **12a**, obtained by the PA-catalyzed resolution of (2*R**,3*S**)-**11a** were assigned (2*S*,3*R*) and (2*R*,3*S*) absolute configurations, respectively. Similarly, the resultant products of the enzymatic hydrolysis of (2*R**,3*R**)-**11b** are (2*S*,3*S*)-**12b** and (2*R*,3*R*)-**13b**.

Conclusions

In summary, we have demonstrated that biomimetic transamination of the commercially available ethyl 2-methyl-3-keto-4,4,4-trifluorobutyrate (**4**) with benzylamine provides a simple access to the 2-methyl-3-amino-4,4,4-trifluorobutanoic acids, a hitherto unknown biologically relevant β-amino acid. In sharp contrast to the α-unsubstituted β-keto carboxylic esters the transamination of α-methyl β-keto carboxylic ester **4** proceeds under mild reaction conditions due to relative instability of the intermediate (*Z*)-enamine **6**. Diastereoselectivity of the process was found to be controlled by the nature of the base-catalyst allowing for a stereodivergent preparation of (2*R**,3*S**)-**8a** and (2*R**,3*R**)-**8b** diastereomers as a dominant reaction product. Preparation of all four diastereo- and enantiomerically pure optical isomers of the 2-methyl-3-amino-4,4,4-trifluorobutanoic acid was effectively accomplished by PA-catalyzed resolution of the corresponding diastereomerically pure *N*-phenylacetyl derivatives.

Very simple set of reactions, application of inexpensive reagents and mild reaction conditions would render this chemoenzymatic approach to the biologically interesting α,β-disubstituted fluorinated β-amino acids methodologically useful alternative for the development of more general and practical process in the future.

Experimental Section

General. For standard laboratory praxis and techniques see related paper, ref 11d. Unless otherwise indicated, ^{1}H, ^{19}F, and ^{13}C NMR spectra were taken in $CDCl_3$ solutions at 299.95, 282.24, and 75.42 MHz, respectively. Chemical shifts refer to tetramethylsilane (TMS) and $CFCl_3$ as the internal standards. Unless otherwise stated, R_f values were taken using *n*-hexane/ethyl acetate (4:1) as an eluting system. Chiral HPLC analyses on free amino acids 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 Nucleosil Chiral [(L)-Hydroxy-Pro] (250 × 4.0 mm), Macherey-Nagel, Germany. Eluent: 5.0 mM $CuSO_4$, flow rate 0.5 mL/min, 35 °C, detection at 235 nm; as reported previously, ref 17. Optical rotations were measured using a Perkin-Elmer 241 polarimeter. Yields refer to isolated yields of products of greater than 95% purity as estimated by capillary GC and/or ^{1}H and ^{19}F NMR spectrometry. All new compounds were characterized by ^{1}H, ^{19}F, ^{13}C NMR, and elemental analysis. Melting points were determined in open capillaries and are uncorrected. Penicillin acylase (EC 3.5.1.11) from *E. coli* was used in soluble form as described previously.[24] Active enzyme concentration was measured by the method developed for titration of PA active centers.[27] Ethyl 2-methyl-3-keto-4,4,4-trifluorobutyrate (**4**) is commercially available from PCR (USA).

Starting compounds **6** and **7** were prepared by the direct condensation, an azeotropic method, between the ethyl 2-methyl-3-keto-4,4,4-trifluorobutyrate (**4**) and benzylamine. The procedure described in ref 12e were followed except for compound **4** was used in the place of the 3-keto-4,4,4-trifluorobutyrate. The resultant mixture was chromatographed, using *n*-hexane/ethyl acetate 50/3 as an eluent, to give enamine **6**, as an individual compound, and ketimine **7** in a mixture with Schiff bases **8a** and **8b**.

Ethyl 2-methyl-3-(*N*-benzylamino)-4,4,4-trifluorocrotonate (6): 15%, R_f 0.45. ^{1}H NMR δ 1.27 (t, 3H, J = 7.2 Hz), 1.94 (q, 3H, J = 3.3 Hz), 4.16 (q, 2H, J = 7.2 Hz), 4.35 (d, 2H, J = 6.6 Hz), 7.24−7.37 (m, 5H), 8.70 (br m, 1H). ^{19}F NMR δ −59.56 (q, J = 3.3 Hz). ^{13}C NMR δ 12.63 (q, J_{CF} = 3.7 Hz), 14.34 (s), 49.98 (q, J_{CF} = 4.0 Hz), 60.61 (s), 100.34 (q, J_{CF} = 5.5 Hz), 122.16 (q, J_{CF} = 280.2 Hz), 127.58 (s), 128.77 (s), 138.84 (s), 146.01 (q, J_{CF} = 29.3 Hz), 170.72 (s). Anal. Calcd for $C_{14}H_{16}F_3NO_2$: C, 58.53; H, 5.61; N, 4.88; F, 19.84. Found: C, 58.61; H, 5.66; N, 4.93; F, 19.71.

Ethyl 2-methyl-3-(*N*-(1-phenylethylidene)imino)-4,4,4-trifluorobutyrate (7): 53% (as a mixture with Schiff bases **8a** and **8b**; ratio: 5.76/1.00/1.12, respectively), R_f 0.30. ^{1}H NMR δ 1.22 (t, 3H, J = 7.2 Hz), 1.48 (dq, 3H, J = 7.2 Hz, 0.9 Hz), 3.90 (q, 1H, J = 7.2 Hz), 4.15 (q, 2H, J = 7.2 Hz), 4.75 (AB, 2H, J = 14.4 Hz), 7.26−7.38 (m, 5H). ^{19}F NMR δ −70.69 (s). Anal. Calcd for $C_{14}H_{16}F_3NO_2$: C, 58.53; H, 5.61; N, 4.88; F, 19.84. Found: C, 58.74; H, 5.69; N, 4.95; F, 19.69.

The base-catalyzed isomerizations of compounds **6**/**7** to the Schiff bases **8a** and **8b** were accomplished under the conditions listed in Table 1. Progress of the reactions was monitored by

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^{19}F NMR, and upon completion, any undissolved solid was removed by filtration. The targeted products, Schiff bases **8a** and **8b**, were isolated by column chromatography. Yields are given in Table 1. When the reactions were run in solutions of TEA, or diethylamine, the corresponding base was evaporated *in vacuo* prior to the chromatography.

Preparative experiments to afford diastereomer (2*R**,3*S**)-**8a** or (2*R**,3*R**)-**8b**, as a dominant product, are exemplified by entries 2 and 8 (Table 1), respectively.

Ethyl 2-methyl-3-(*N*-benzylideneamino)-4,4,4-trifluorobutyrate (8): as 1.7/1 mixture of (2*R**,3*S**)-**8a** and (2*R**,3*R**)-**8b** diastereomers (prepared in TEA). R_f 0.31; (2*R**,3*S**)-**8a**: ^{1}H NMR δ 1.12 (t, 3 H, J = 7.2 Hz), 1.39 (dq, 3 H, J = 7.2 Hz, 1.5 Hz), 3.15 (quin, 1 H, J = 7.2 Hz), 3.95 (dq, 1 H, J = 8.5 Hz, 7.2 Hz), 4.04 (q, 2 H, J = 7.2 Hz), 7.39−7.46 (m, 3 H), 7.75−7.82 (m, 2 H), 8.30 (s, 1 H). ^{19}F NMR δ −70.59 (d, J = 8.5 Hz); (2*R**,3*R**)-**8b**: ^{1}H NMR δ 1.24 (dq, 3 H, J = 7.2 Hz, 0.6 Hz), 1.26 (t, 3 H, J = 7.2 Hz), 3.13 (m, 1 H), 4.11 (m, 1 H), 4.14, 4.20 (AB, 2 H, J = 7.2 Hz, J_{AB} = 10.8 Hz), 7.39−7.46 (m, 3 H), 7.75−7.82 (m, 2 H), 8.37 (s, 1 H). ^{19}F NMR δ −73.35 (d, J = 8.7 Hz). Anal. Calcd for $C_{14}H_{16}F_3NO_2$: C, 58.53; H, 5.61; N, 4.88; F, 19.84. Found: C, 58.74; H, 5.67; N, 4.71; F, 19.80.

Ethyl 2-methyl-3-amino-4,4,4-trifluorobutyrate (9), hydrochloride: as 1.7/1 mixture of (2*R**,3*S**)-**9a** and (2*R**,3*R**)-**9b**. The starting mixture of Schiff bases (2*R**,3*S**)-**8a** and (2*R**,3*R**)-**8b**, ratio 1.7/1, respectively, 6.5 g (22.6 mmol) was dissolved in 25 mL of ether and 10 mL of 0.5 N HCl was added under stirring at ambient temperature. Progress of the hydrolysis was monitored by TLC and upon completion (1 h) aqueous layer was separated, washed with ether, and evaporated *in vacuo* to give 4.9 g (91.9%) of product **9**. (2*R**,3*S**)-**9a**: ^{1}H NMR (CD_3CN) δ 1.25 (t, 3 H, J = 7.2 Hz), 1.44 (d, 3 H, J = 7.2 Hz), 3.33 (qd, 1 H, J = 7.2 Hz, 4.2 Hz), 4.21 (q, 2 H, J = 7.2 Hz), 4.54 (m, 1 H). ^{19}F NMR (CD_3CN) δ −68.89 (d, J = 8.5 Hz); ^{13}C NMR (CD_3CN) δ 12.40 (s), 13.36 (s), 37.48 (s), 52.46 (q, J_{CF} = 30.8 Hz), 62.08 (s), 127.30 (q, J_{CF} = 281.7 Hz), 171.55 (s). (2*R**,3*R**)-**9b**: ^{1}H NMR (CD_3CN) δ 1.27 (t, 3 H, J = 7.2 Hz), 1.45 (d, 3 H, J = 7.2 Hz), 3.23 (qd, 1 H, J = 7.2 Hz, 4.8 Hz), 4.21 (m, 2 H), 4.54 (m, 1 H). ^{19}F NMR (CD_3CN) δ −70.03 (d, J = 8.5 Hz). ^{13}C NMR (CD_3CN) δ 12.32 (s), 13.39 (s), 37.16 (s), 53.45 (q, J_{CF} = 31.1 Hz), 62.07 (s), 123.83 (q, J_{CF} = 281.7 Hz), 171.48 (s).

2-Methyl-3-amino-4,4,4-trifluorobutyric acid (α-methyl-β-(trifluoromethyl)-β-alanine) (10): as 1.7/1 mixture of (2*R**,3*S**)-**10a** and (2*R**,3*R**)-**10b** diastereomers. Hydrochloride **9** [as 1.7/1 mixture of (2*R**,3*S**)-**9a** and (2*R**,3*R**)-**9b** diastereomers] (4.5 g, 19.1 mmol) was dissolved in 25 mL of 2 N HCl, and the resultant solution was heated at 50 °C for 1 week. The reaction mixture was evaporated *in vacuo* to dryness. Standard Dowex-50 column chromatography of the crystalline residue afforded 2.65 g (81.1%) of free amino acid **10**. Mp 163−7 °C; (2*R**,3*S**)-**10a**: ^{1}H NMR (CD_3CN) δ 1.13 (dq, 3 H, J = 7.2 Hz, 1.2 Hz), 2.62 (dq, 1 H, J = 7.2 Hz, 5.1 Hz), 4.17 (dq, 1 H, J = 7.8 Hz, 5.1 Hz). (2*R**,3*R**)-**10b**: ^{1}H NMR (CD_3CN) δ 1.08 (d, 3 H, J = 7.3 Hz), 2.59 (m, 1 H), 3.99 (m, 1H). Anal. Calcd for $C_5H_8F_3NO_2$: C, 35.10; H, 4.71; N, 8.19; F, 33.31. Found: C, 35.17; H, 4.73; N, 8.08; F, 33.15.

(2*R,3*S**)-2-Methyl-3-amino-4,4,4-trifluorobutyric acid (10a) and (2*R**,3*R**)-2-Methyl-3-amino-4,4,4-trifluorobutyric acid (10b):** obtained in 41% and 35% yield, respectively, by crystallization, from acetone/Et_2O, of the corresponding mixtures of amino acids containing diastereomers (2*R**,3*S**)-**10a** or (2*R**,3*R**)-**10b** as a dominant product. Diastereomeric purity of amino acids (2*R**,3*S**)-**10a** and (2*R**,3*R**)-**10b** was determined by NMR and HPLC analysis.

N-Phenylacetyl derivatives (2*R**,3*S**)-**11a** and (2*R**,3*R**)-**11b** were prepared by phenylacetylation of diastereomerically pure amino acids (2*R**,3*S**)-**10a** or (2*R**,3*R**)-**10b**, respectively, with phenylacetyl chloride in the presence of potassium bicarbonate in aqueous acetone at −5 °C, according to the general procedure for phenylacetylation of β-perfluoroalkyl-β-amino acids.[12d]

(2*R,3*S**)-2-Methyl-3-(*N*-phenylacetylamino)-4,4,4-trifluorobutyric acid (11a):** 87%, mp 139−143 °C. ^{1}H NMR δ 1.21 (dq, 3 H, J = 7.2 Hz, 0.6 Hz), 2.68 (d, 2 H, J = 2.1 Hz), 2.96 (dq, 1 H, J = 7.2 Hz, 4.5 Hz), 4.89 (m, 1 H), 7.33 (m, 5 H), 7.62 (m, 1H). Anal. Calcd for $C_{13}H_{14}F_3NO_3$: C, 53.98; H, 4.88; N, 4.84; F, 19.71. Found: C, 54.05; H, 4.93; N, 4.80; F, 19.57.

(2*R,3*R**)-2-Methyl-3-(*N*-phenylacetylamino)-4,4,4-trifluorobutyric acid (11b):** 82%, mp 148−151 °C. 1.82 (d, 3 H, J = 7.2 Hz), 2.34 (dq, 1 H, J = 8.1 Hz, 7.2 Hz), 3.63 (bs, 2 H), 5.15 (quint, 1 H, J = 8.1 Hz), 7.28 (m, 1 H), 7.31 (m, 5H). Anal. Calcd for $C_{13}H_{14}F_3NO_3$: C, 53.98; H, 4.88; N, 4.84; F, 19.71. Found: C, 54.01; H, 4.89; N, 4.82; F, 19.65.

Enzymatic Hydrolysis of *rac*-*N*-Phenylacetyl Derivatives (2*R,3*S**)-11a and (2*R**,3*R**)-11b. 2-Methyl-3-amino-4,4,4-trifluorobutyric Acids: (2*S*,3*R*)-13a, (2*R*,3*R*)-13b, (2*R*,3*S*)-14a, and (2*S*,3*S*)-14b.** In a typical procedure, *N*-phenylacetyl derivatives (2*R**,3*R**)-**11a** and (2*R**,3*S**)-**11b** (1 mmol) were dissolved in 5 mL of water, and after adjusting of pH of resulting solutions with 5% NH_4OH to 7.5, 0.15 mL of 10^{-6} M penicillin acylase (EC 3.5.1.11) from *E. coli* was added. The reaction mixtures were stirred at room temperature, and the course of hydrolysis was monitored by consumption of 5% NH_4OH and was stopped at an appropriate point to obtain 50% conversion of starting material. Then, the pH of the solutions were adjusted to 2 with a 1 M HCl and extracted with ethyl acetate to give enzymatically unconverted *N*-phenylacetyl derivatives (2*R*,3*S*)-**12a** and (2*S*,3*S*)-**12b** (organic layer) and amino acids (2*S*,3*R*)-**13a** and (2*R*,3*R*)-**13b** (aqueous layer). Aqueous phases were chromatographed on Dowex-50 to give (87% yield) free (2*S*,3*R*)-2-methyl-3-(trifluoromethyl)-3-aminobutanoic acid (**13a**) ([α]$^{25}_D$ +18.7, *c* 0.3, H_2O) and, from another experiment, (2*R*,3*R*)-**13b** ([α]$^{25}_D$ +20.9, *c* 0.5, H_2O) (85% chemical yield).

The ethyl acetate extracts were combined, dried with $MgSO_4$, and evaporated. The resultant *N*-phenylacetyl derivatives (2*R*,3*S*)-**12a** and (2*S*,3*S*)-**12b**, checked by NMR (>95% purity), were dissolved each in 6 N HCl and heated at 70 °C for 11 h. The reaction mixture was evaporated *in vacuo* to dryness. The residue was dissolved in water and washed with ethyl acetate. Free amino acids (2*R*,3*S*)-**14a** ([α]$^{25}_D$ −18.1, *c* 0.1, H_2O) (67% yield) and (2*S*,3*S*)-**14b** ([α]$^{25}_D$ −20.3, *c* 0.1, H_2O) (70% chemical yield) were isolated from the aqueous phase using Dowex-50 column chromatography.

NMR spectra of thus obtained amino acids **13a**, **13b** and **14a**, **14b** are identical to that of *rac*-**10a** and **10b** (*vide versa*). Chiral HPLC analysis of crude amino acids **13a**, **13b** and **14a**, **14b** shown their optical purity is >95%.

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