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Chemo-Enzymatic Approach to the Synthesis of Each of the Four Isomers of α-Alkyl-β-Fluoroalkyl-Substituted β-Amino Acids

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Abstract: Starting from easily available ethyl 2-methyl-4,4,4-trifluoroacetoacetate and benzylamine each of the four stereoisomers of α-methyl-β-trifluoromethyl-β-alanine have been synthesized in optically pure form *via* stereocontrolled chemo-enzymatic procedure including diastereoselective base-catalyzed [1,3]-proton shift reaction and enantioselective penicillin acylase-catalyzed resolution.

The recent upsurge of interest in the carbapenem antibiotics[2] has been accompanied by a great deal of attention to the synthesis of various α-,β-disubstituted β-amino acids[3] which are one of the key structural units in the penem skeleton. However, among the large number of α-,β-disubstituted β-amino acids described, fluorine-containing ones are practically unknown.[4,5] Our recent results on biomimetic [1,3]-proton shift reaction[6] and biocatalytic resolution of β-amino acids[7] prompted us to couple these two methods in one chemo-enzymatic approach that includes stereoselective synthesis of desirable diastereomer and next, biocatalytic resolution of pure diastereomer into the pair of enantiomers. We report here our preliminary results on the application of this methodology to a synthesis of each of the four stereoisomers of hitherto unknown α-methyl-β-trifluoromethyl-β-alanine (Scheme 1).

Previously, we have reported[6a] that the *N*-benzyl imine/*N*-benzyl enamine mixture **2**, prepared from ketoester **1**[8] and benzylamine, on the treatment with triethylamine easily underwent [1,3]-proton transfers to give in a yield of 94% the pair of diastereomers **3a,b** in the 37:63 ratio. Further experiments revealed that this ratio is uninfluenced by the solvent, reaction temperature, and the time of exposition with triethylamine, but sensitive to the nature of base employed. Thus, we have found that catalysis of the isomerization with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) results in the nearly the same ratio of diastereomers **3a,b**,[9] but with domination of the opposite diastereomer. This stereochemical result (ratio **3a/3b** as 65/35) was obtained also by the action of catalytic amount (10 mol%) of DBU on the mixture **3a** and **3b** (ratio 37/63) formed in the

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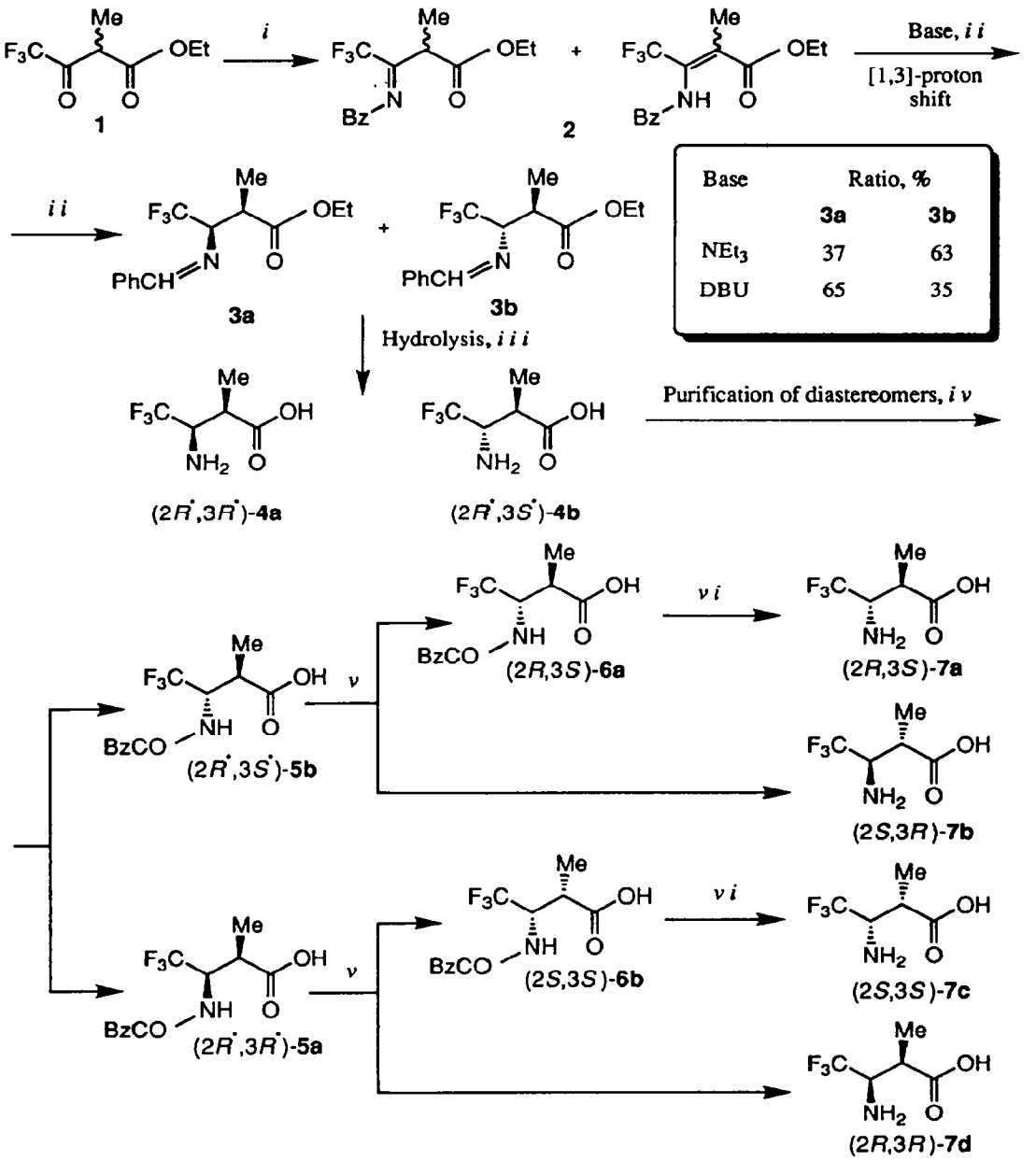
Scheme 1



Base	Ratio, %	
	3a	**3b**
NEt_3	37	63
DBU	65	35

Reagents and conditions: *i*, benzylamine, benzene, Dowex-50, reflux; *i i*, triethylamine or DBU, 20-30 °C; *i i i*, 2 N HCl, Et_2O, 20-25 °C, 2 hr., then 6 N HCl, 100 °C, 6 hr.; *i v*, water-acetone, phenylacetyl chloride, $KHCO_3$, –5° C, 4 hr.; *v*, penicillin acylase, 20-23 °C, pH 7.5, 4-5 hr.; *v i*, 6 N HCl, 70 °C, 12 hr.

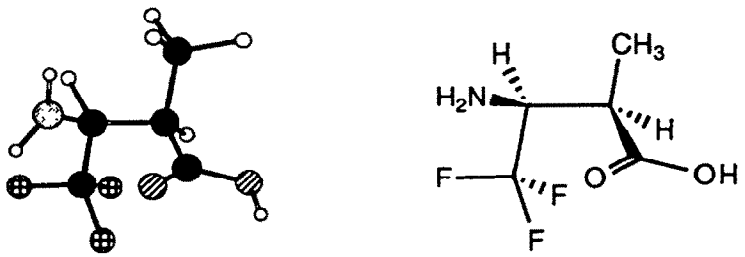


Figure 1. X-Ray structure of (2*R**, 3*S**)-2-methyl-3-trifluoromethyl-3-aminobutanoic acid.

triethylamine-catalyzed reaction. Mixtures of diastereomers **3a,b**[10] were easily hydrolyzed (Scheme 1) to give free amino acids **4a,b** which were isolated in diastereomerically pure form by recrystallizations of corresponding mixtures containing **4a** or **4b** as a dominant diastereoisomer respectively.[11] The relative stereochemistry at C-2 and C-3 of the amino acids obtained was determined by X-ray analysis[12] (Figure 1) of diastereoisomer **4b** which showed its (2*R**,3*S**)-configuration. Next, *N*-phenylacetyl derivatives[13] (2*R**,3*R**)-**5a** and (2*R**,3*S**)-**5b** were synthesized with excellent isolated yields *via* Schotten-Baumann procedure by treatment of water-acetone solutions of β-amino acids **4a** and **4b** in the presence of potassium bicarbonate with phenylacetyl chloride at low temperature (-5° C) (Scheme 1). The preparative enzymatic resolution of substrates **5a** and **5b** is outlined in the Scheme 1. In a typical procedure, *N*-phenylacetyl derivatives (2*R**,3*R**)-**5a** and (2*R**,3*S**)-**5b** (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[14] was added. The 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 **6a,b** (organic layer) and amino acids **7b,d** (aqueous layer). Aqueous phases were chromatographied on Dowex-50 to give free (2*S*,3*R*)-2-methyl-3-trifluoromethyl-3-aminobutanoic acid (**7b**) ($[\alpha]_D^{25}$ +18.7, *c* 0.3, H_2O) and, from another experiment, (2*R*,3*R*)-isomer **7d** ($[\alpha]_D^{25}$ +20.9, *c* 0.5, H_2O). Chiral HPLC analysis of crude amino acids **7b,d** have shown their optical purity is at least 96-98% (the sensitivity limit of the method[7]), that suggest excellent enantioselectivity of the enzyme. The two rest optical isomers of α-methyl-β-trifluoromethyl-β-alanine (2*R*,3*S*)-**7a** ($[\alpha]_D^{25}$ -18.1, *c* 0.1, H_2O) and (2*S*,3*S*)-**7c** ($[\alpha]_D^{25}$ -20.3, *c* 0.1, H_2O) were prepared by chemical hydrolysis of enzymatically unconverted *N*-phenylacetyl derivatives **6a** and **6b** (Scheme 1). Since it has been shown that penicillin acylase is highly enantioselective towards (*L*)-enantiomers of various amino acids and their derivatives[15] including β-polyfluoro-alkyl-β-amino acids[7] and the rate constants of enzymatic hydrolysis is practically uninfluenced by the presence of α-methyl group[16] as well, we have attributed (R)-absolute configuration at C-3 for biocatalytically prepared β-amino acids **7b** and **7d**, and consequently (2*R*,3*S*) for **7a**, (2*S*,3*R*) for **7b**, (2*S*,3*S*) for **7c**, and (2*R*,3*R*) for **7d**.

The full scope of the methodology described is currently under the active investigation.

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8 Was prepared with 78% isolated yield by condensation of methyl trifluoroacetate with methyl propionate under the action of sodium hydride.

9 Yield 64%. Much lower yield in the DBU-catalyzed isomerization can explained by partial decomposition of products 3a,b through dehydrofluorination under the action of such a strong base as DBU.

10 ^{1}H NMR (δ, $CDCl_3$) for *N*-benzylidene derivatives **3a**,**b**: (2*R**,3*R**)-**3a**, 1.39 (dq, *J* = 7.2 Hz, 1.5 Hz, 3 H), 3.16 (m, 1 H), 3.58 (s, 3 H), 3.95 (dq, *J* = 8.7 Hz, 7.2 Hz, 1 H), 7.39-7.79 (m, 5 H), 8.30 (s, 1 H). (2*R**,3*S**)-**3b**, 1.24 (d, *J* = 7.2 Hz, 3 H), 3.15 (m, 1 H), 3.71 (s, 3 H), 4.11 (qu, *J* = 7.2 Hz, 1 H), 7.40-7.81 (m, 5 H), 8.37 (s, 1 H).

11 ^{1}H NMR (δ, $CDCl_3$) for amino acids **4a**,**b**: (2*R**,3*R**)-**4a**, 1.08 (d, *J* = 7.3 Hz, 3 H), 2.59 (m, 1 H), 3.99 (m, 1H). (2*R**,3*S**)-**4b**, 1.13 (dq, *J* = 7.2 Hz, 1.2 Hz, 3 H), 2.62 (dq, *J* = 7.2 Hz, 5.1 Hz, 1 H), 4.17 (dq, *J* = 7.8 Hz, 5.1 Hz, 1 H).

12 Crystals of amino acid **4b** were grown from water-ethanol solution. Crystal data for **4b**: $C_5H_8F_3NO_2$, space group PBCN (#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.

13 ^{1}H NMR (δ, $CDCl_3$) for *N*-phenylacetyl derivatives **5a**,**b**: (2*R**,3*R**)-**5a**, 1.82 (d, *J* = 7.2 Hz, 3 H), 2.34 (dq, *J* = 8.1 Hz, 7.2 Hz, 1 H), 3.63 (bs, 2 H), 5.15 (qu, *J* = 8.1 Hz, 1 H), 7.28 (m, 1 H), 7.31 (m, 5H). (2*R**,3*S**)-**5b**, 1.21 (dq, *J* = 7.2 Hz, 0.6 Hz, 3 H), 2.96 (dq, *J* = 7.2 Hz, 4.5 Hz, 1 H), 2.68 (d, *J* = 2.1 Hz, 2 H), 4.89 (m, 1 H), 7.33 (m, 5 H), 7.62 (m, 1H).

14 Penicillin acylase (EC 3.5.1.11) from *Escherichia coli* was used. See ref. 15 (b).

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