ELSEVIER

Journal of Fluorine Chemistry 69 (1994) 199–205

JOURNAL OF FLUORINE CHEMISTRY

Fluorine-containing amino acids*

V.P. Kukhar

Institute of Bioorganic Chemistry and Petrochemistry, Ukrainian Academy of Sciences, Murmanskaya Str., 1, Kiev, 253660, Ukraine

Received 13 January 1993; accepted 1 August 1993

Abstract

This mini-review covers Ukrainian studies on the synthesis of chiral fluorine-containing amino acids by catalytic reduction, perfluoroalkylation of hydroxy-containing amino acids and enzymatic resolution of racemic mixtures of fluorine-containing amino acids.

Keywords: Fluorine-containing amino acids; Synthesis; Catalytic reduction; Perfluoroalkylation; Enzymatic resolution

1. Introduction

The chemistry of fluoroorganic compounds has been developed at the Ukrainian Academy of Sciences over the past 50 years. During this time, some major successes have been achieved in the elaboration of new methods of synthesis of organofluorine compounds, in the construction of new fluorine-containing groups and in the investigation of their properties.

It is known that organofluorine chemistry is applicable over many branches of chemistry and industry, and we expect to see a very quick penetration of organofluorine chemistry into bioorganic chemistry and biochemistry [1].

In the laboratory of Fine Organic Synthesis at the Institute of Bioorganic Chemistry and Petrochemistry of the Ukrainian Academy of Sciences the investigation of the synthesis and properties of fluorine-containing compounds to natural products is being developed. Some years ago we began a programme on the investigation of fluorine-containing amino acids. First we developed general methods for introducing fluorine or fluorine-containing substituents into amino acid structures [2–4]. The main object of present studies is the stereospecific preparation of fluorine-containing amino acids, i.e. obtaining the enantiomers of such compounds.

2. Results and discussion

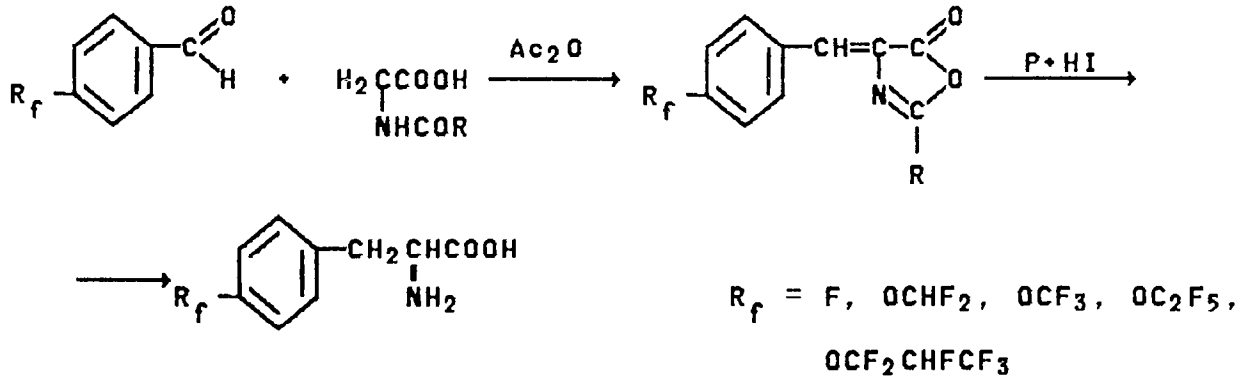
Investigations of fluorine-containing amino acids have brought a number of interesting results. One of the most interesting is the biological activity of β-fluorine-containing amino acids. These compounds were found to be highly selective and potent inhibitors of pyridoxal phosphate-dependent enzymes via a 'suicide-type' mechanism [5]. The first step of the reaction consists of interaction between β-fluorine-containing amino acid and the coenzyme, i.e. pyridoxal phosphate. After formation of the imine, the β-fluorine atom is activated towards anion elimination, a process which occurs simultaneously with enzyme action. Such a transformation yields a Michael acceptor in which the double bond reacts easily with a nucleophilic centre or the NH, OH or SH groups of the enzyme to induce irreversible inhibition of the enzyme. Thus, β-fluoro amino acids are not direct inhibitors; such inhibition is generated by the enzyme during the reaction process.

Biochemical studies need stereoisomeric compounds. At present we are investigating methods of obtaining chiral amino acids containing fluorine atoms. For this we use asymmetric synthesis, the resolution of racemates and the modification of natural amino acids with organofluorine synthons.

Phenylalanine derivatives with fluorinated groups can be prepared by the azalactone method (Erlenmaier method). In this way fluorine-containing benzaldehydes have been introduced into reaction with *N*-acylglycine,

*Dedicated to Professor L.M. Yagupolskii on the occasion of his 70th birthday.

SSDI 0022-1139(94)03131-I

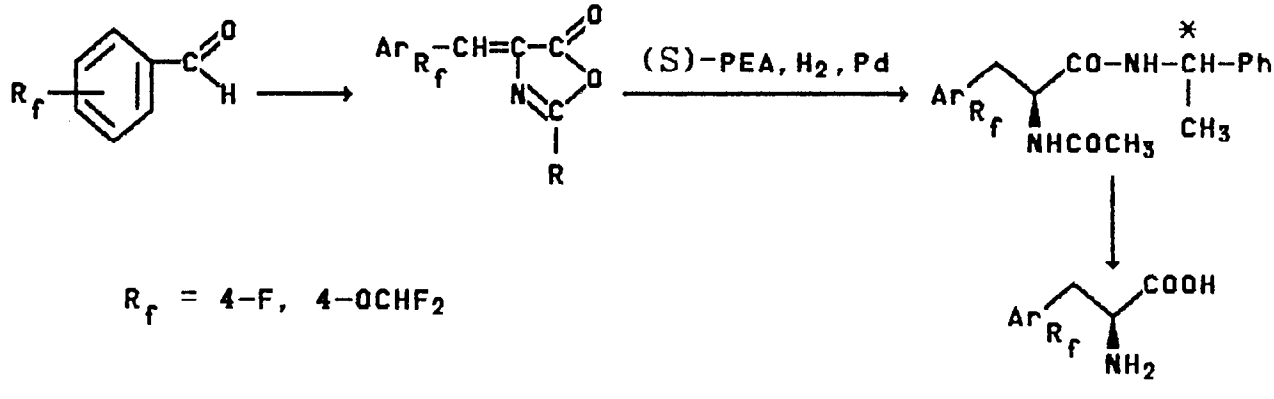


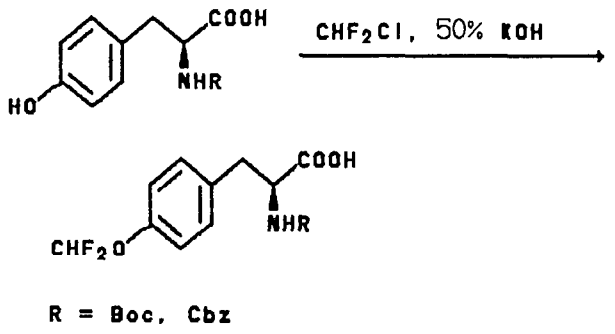
and then the obtained oxazolones were reduced to amino acids [6].

Using this method we have obtained racemic mixtures of amino acids, and in collaboration with the laboratory of Prof. E. Klabunovskyi we have studied the catalytic reduction of the azalactone of *p*-fluoroacetamidocinnamic acid [7]. The catalytic reduction of the azalactones was carried out with hydrogen in dimethoxyethane or ^{i}PrOH solution in the presence of palladium chloride and (*S*)-(−)-α-phenylethylamine (PEA) and triethylamine as additives. The phenylethylamide of *N*-acetyl-*p*-fluorophenylalanine was obtained in quantitative yield, predominantly as the (*S*,*S*)-diastereomer which after crystallization and hydrolysis with hydrochloric acid gave the pure (*S*)-(−)-*p*-fluorophenylalanine. When dimethoxyethane was used as the solvent in the reaction, the diastereomeric excess of the (*S*,*S*)-diastereomer was 28% while in isopropanol this increased to 46%. The hydrogenation and aminolysis stages could be separated without decreasing in the yield of (*S*,*S*)-diastereomer formed in the reaction.

We have applied this method of reductive aminolysis to the synthesis of the phenylethylamides of *N*-acyl-*p*-difluoromethoxyphenylalanine. The highest yield of (*S*,*S*)-diastereomer was obtained in the reaction conducted in t-butanol solution (47% d.e.). The use of the two-step procedure, i.e. hydrogenation followed by aminolysis of the saturated azalactone with (*S*)-phenylethylamine, led to a slight improvement in the stereoselectivity and the diastereomeric excess of the (*S*,*S*)-diastereomer increased up to 55% d.e. Unfortunately, hydrolysis of the amide with hydrochloric acid proceeded with simultaneous elimination of the difluoromethoxy group and tyrosine formation, and the desired amino acid was not obtained [8].

For synthesis of the optically active fluorine-containing phenylalanines we decided to apply the well-known method of perfluoroalkylation of the phenol groups using (*S*)-tyrosine as the chiral synthon. However, we could not introduce unprotected tyrosine for difluoromethylation with Freon-22 under standard conditions. However, we suggested that protection of the amino group, to give stability in concentrated potassium hydroxide at 70 °C and also the possibility of subsequent deprotection without involving the acid labile difluoromethoxy group, could be used to effect the difluoromethylation of tyrosine. Fortunately, the reactions of *N*-Boc- and *N*-Cbz-(*S*)-tyrosine, containing the protected amino group, with Freon-22 at 70 °C in a mixture of isopropanol and 50% aqueous KOH led to the formation of N-protected *p*-difluoromethoxyphenylalanines in 80%–90% yield [9].

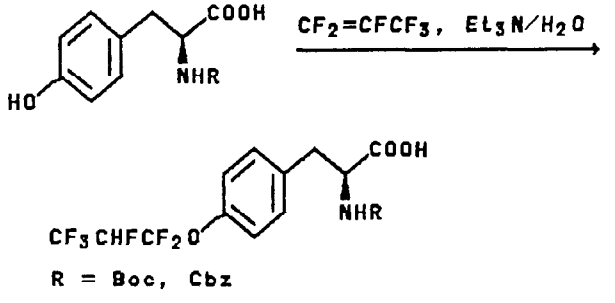




Removal of the Boc-protective group could be easily achieved by action with trifluoroacetic acid at 20 °C, resulting in (*S*)-*p*-difluoromethoxyphenylalanine being obtained in 80% yield. On treatment of the N-protected amino acids with diazomethane, the methyl esters of these amino acids were obtained.

In a similar manner, the difluoromethylation of both hydroxy groups in 3,4-dihydroxyphenylalanine was effected, although the corresponding amino acid was obtained in somewhat lower yield.

The synthesis of perfluoroalkyl esters of (*S*)-tyrosine was realized by reaction between the N-protected amino acid and hexafluoropropylene in aqueous triethylamine [9].



We have found that the reaction of trifluorochlorobromoethane with phenols under phase catalysis conditions in the presence of alkali yields polyhalogenoethylaryl ethers [10]. The reaction proceeds via dehydrofluorination and the subsequent addition of fluoro-olefins to phenol. Introduction of *S*-tyrosine into this reaction led to the corresponding (*S*)-4-(2-bromo-2-chloro-1,1-difluoroethoxy)-*N*-Boc-phenylalanine [9]. Subsequent deprotection with trifluoroacetic acid using standard methods gave unprotected polyfluoroalkoxy-containing (*S*)-phenylalanines. The introduction of fluorine-containing groups in (*S*)-tyrosine proceeds without racemization.

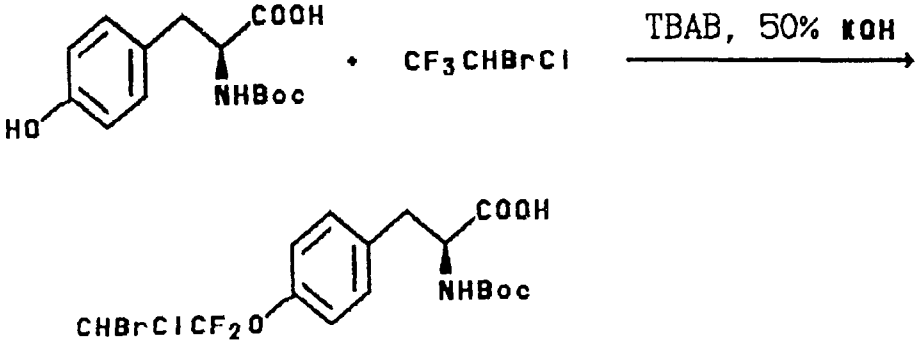
Using HPLC methods, we have been able to establish the lipophilicity of some fluorine-containing groups introduced into phenylalanines. From the data listed below it is seen that the introduction of fluorinated groups provides the possibility of widely varying the lipophilicity, the latter increasing with an increase in the fluorine content [11].

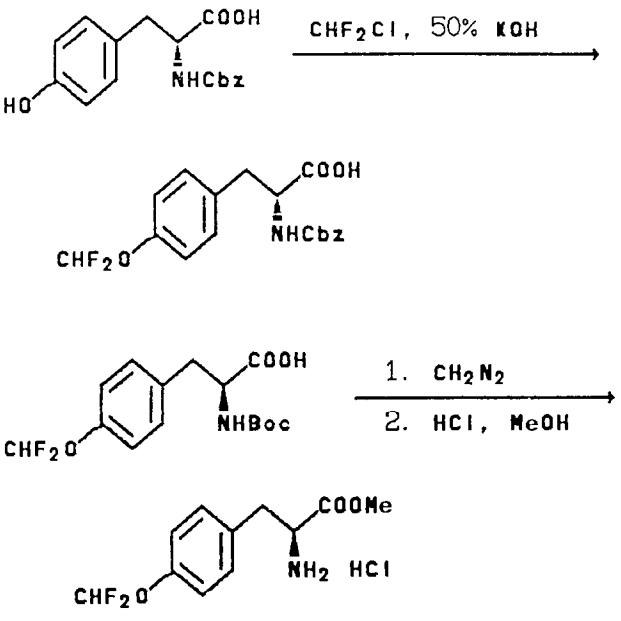
CF_3CHFCF_2O	C_2F_5	CF_3S	CF_3O
1.90	1.80	1.43	1.05

CHF_2S	CF_3CH_2O	$CHClBrCF_2O$	CHF_2O	F
0.95	0.93	0.69	0.57	0.14

In our opinion, of the synthesized phenylalanines, compounds containing CHF_2O groups are the most interesting since this group has a high lipophilicity factor ($\pi_x = 0.57$) and at the same time there is a negligible change in the volume size between the fluorinated analogue and the methoxy group often found in natural biologically active compounds. In addition, this group is readily introduced with high yields of the desired products.

p-Difluoromethoxyphenylalanine has been used by us for the synthesis of fluorine-containing analogues of the virus replication inhibiting peptide Cbz-(D)-(Phe)-(L)-Phe-Gly. For this, the methyl ester of *p*-difluoromethoxy-(L)-*N*-Boc-phenylalanine was obtained by methylation of *p*-difluoromethoxy-(L)-*N*-Boc-phenylalanine with diazomethane and, following deprotection of the Boc group, *p*-difluoromethoxy-(D)-phenylalanine was prepared from N-Cbz-(D)-tyrosine by O-difluoromethylation.





Using these blocks and standard methods of peptide synthesis we have synthesized fluorine-containing analogues of the antivirus peptide.

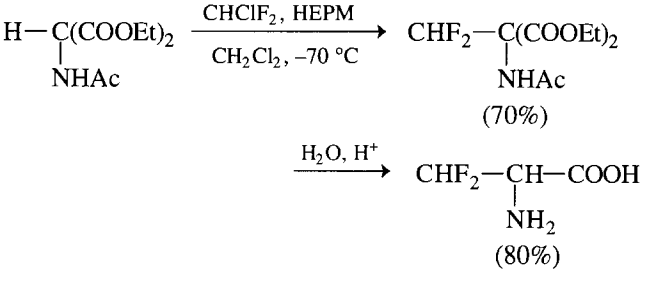
The hydroxyphenyl group is also present in D-α-(4-hydroxyphenyl)glycine which has been used for the preparation of the semi-synthetic penicillins and cephalosporines. We have also achieved the O-polyfluoroalkylation of the *N*-Boc derivative of this amino acid, leading to difluoromethoxy- and hexafluoropropoxyphenylglycines in 50%–60% yield [12].

However during the difluoromethylation reaction the amino acid formed was found to have undergone full racemization, whereas reaction with hexafluoropropylene proceeded without racemization and gave the corresponding D-phenylglycine.

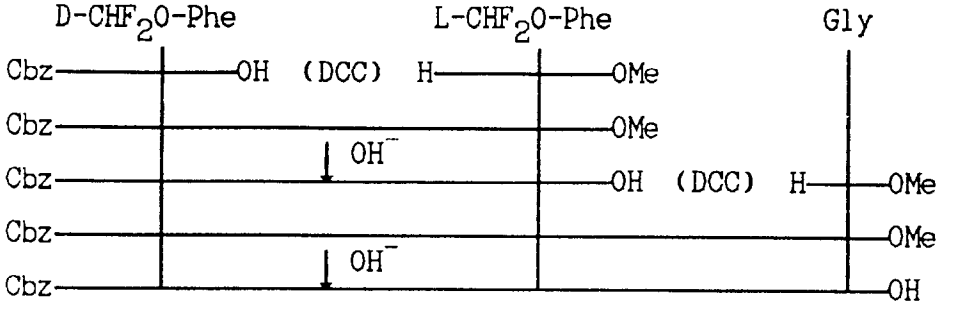
The stereochemical aspects of these reactions have been studied by NMR spectroscopy using the chiral shift reagent $Eu(hfc)_3$ for the methyl esters of fluorinated phenylglycines. Differences in the behaviour of phenylglycines and phenylalanines in the polyfluoroalkylation reaction may be due to the different influences of the phenyl and benzyl groups on the α-proton of the amino acid.

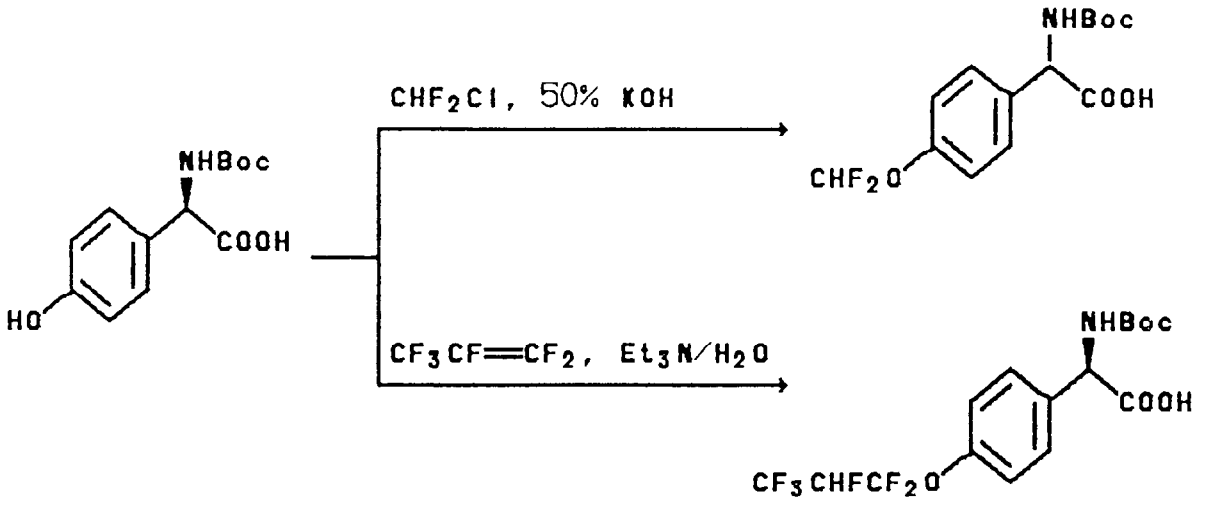
The lower acidity of the alkylhydroxy group in comparison with a phenol hydroxy group prevents the difluoromethylation of alcohols in aqueous solution. However this process may be effected in excess alcohol or in aprotic solvents by the use of 'non-nucleophilic' bases. In collaboration with Prof. Yu. Yagupolsky and A. Kolomietsev, we have used tris(diethylamino)-phosphazomethane as a base which is soluble in most organic solvents. As an application of this strong base, we attempted the O-difluoromethylation of the aliphatic hydroxy-containing amino acid, serine. However, we found that we could not introduce the difluoromethyl group into (L)-*N*-Boc-serine by the action of this base in methylene chloride solution. Only the starting amino acid was isolated from the reaction mixture. Under the same conditions, the methyl ester of (L)-*N*-Boc-serine yielded the dehydroalanine derivative, probably as a result of elimination of the pseudohalogen group CHF_2O from the product of serine difluoromethylation [13].

In contrast to the unsuccessful O-difluoromethylation of serine, the difluoromethylation of diethyl *N*-acetylaminomalonate in the presence of phosphazomethane proceeds in methylene chloride at −70 °C with the formation of the difluoromethyl derivative in 70% yield [14].

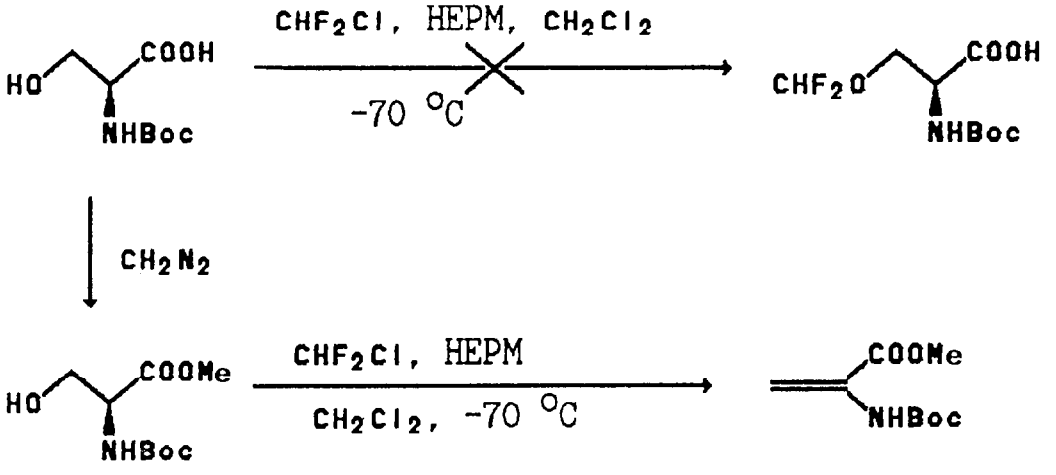


Previously, difluoromethylation of amino malonate derivatives was carried out using lithium diisopropylamide or lithium hexamethyldisilazane with yields of ~30%, underlining the advantages of using the new strong base, phosphazomethane. After hydrolysis of the difluoromethylated ester in an acid medium, the racemic difluoromethylalanine — a highly effective inhibitor of alanine racemase — was obtained in 80% yield.

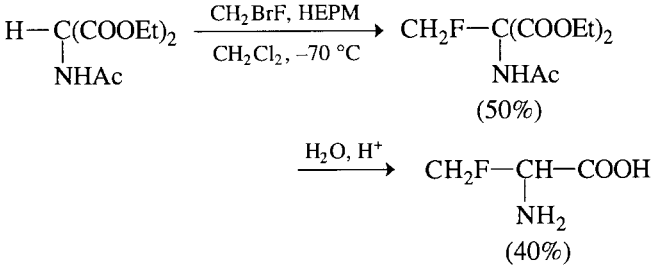




$(Et_2N)_3P{=}NMe$ (HEPM)



Monofluoroalanine is known to be a potent inhibitor of a number of bacterial alanine racemases. A few methods exist for its synthesis. We decided to apply a method similar to the above using bromofluoromethane instead of chlorodifluoromethane; however, the yields were lower for all stages [14].



For the preparation of 3-fluoroalanine, we used the methyl ester of 2-bromo-3-fluoropropionic acid which had previously proved unsuccessful because of the elimination of fluorine atoms. Synthesis of the ester was realized by reaction of the methyl ester of 2,3-dibromopropionic acid with boron trifluoride in the presence of 1–3 mol% $SnCl_4$ as catalyst. The ester was obtained in 70% yield [15].

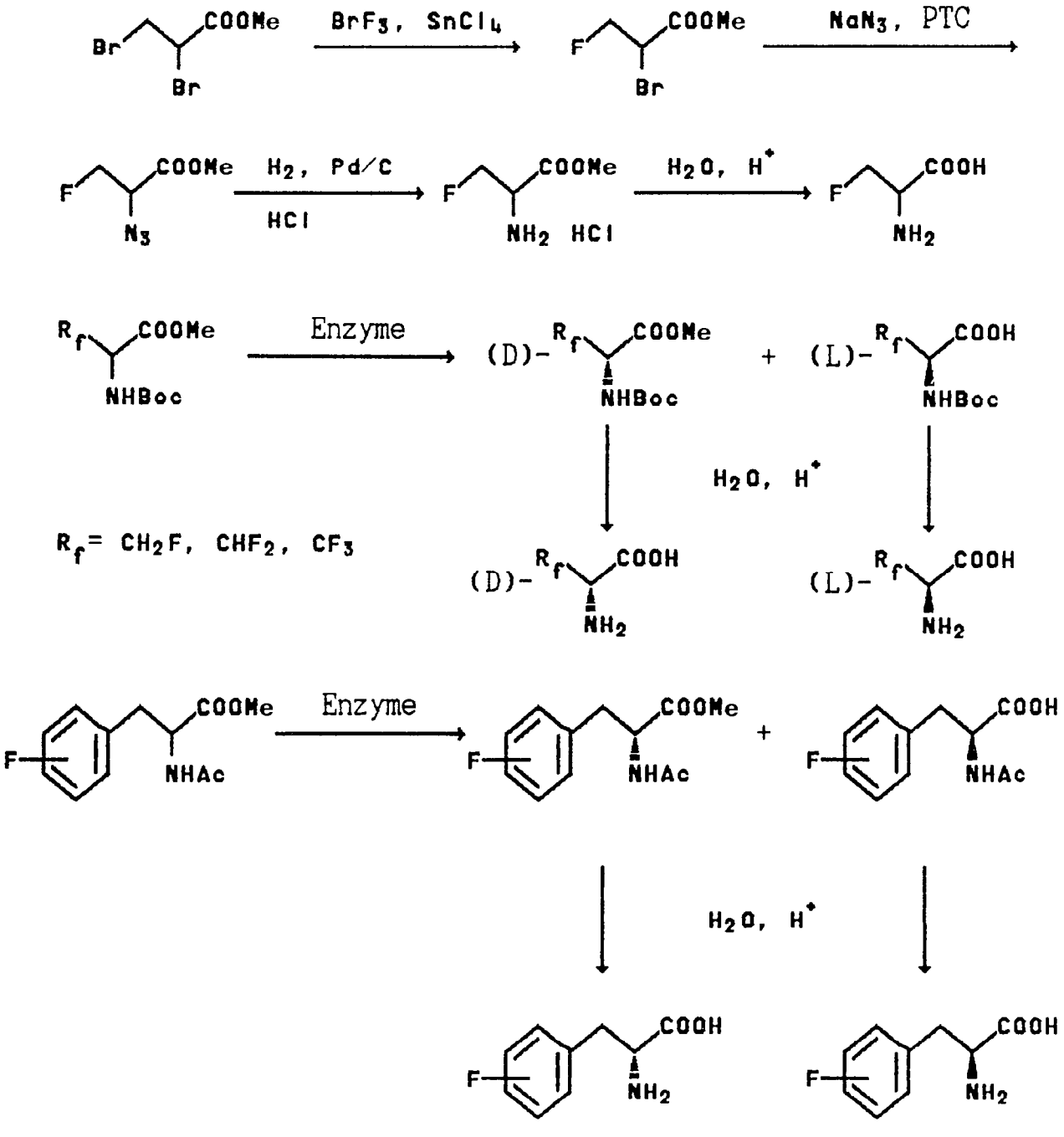
Reaction of the ester with sodium azide in methylene chloride under two-phase conditions led to the methyl ester of 2-azido-3-fluoropropionic acid, which could be reduced by hydrogen with palladium on charcoal to form the methyl ester of 3-fluoroalanine in high yield. To prevent elimination of hydrogen fluoride, the reduction was carried out in a methanol formic acid mixture followed by saturation of the reaction medium with hydrogen chloride. Reduction of azide in the presence of hydrogen chloride proceeds slowly and gives a low yield of the hydrochloride. Employing the usual treatment, i.e. hydrolysis and the action of propylene oxide, deprotected 3-fluoroalanine was obtained as a racemate.

The methyl ester of 3-fluoropropionic acid was converted into the ester of *N*-Boc-3-fluoro-(*R*,*S*)-alanine which we have used for the resolution of enantiomers with the enzyme papain. Papain may be used to stereoselectively hydrolyze the ester bond of the (*R*)-isomer of the racemic mixture at 18–28 °C and a pH of 6.0–7.0 to give a quantitative yield of the pure (*R*)-isomer in 2–3 h [15].

Methylation of the latter with diazomethane gave the corresponding methyl ester. The Boc-protective group could be eliminated from esters of (*S*)- and (*R*)-fluoroalanines by treatment with 5 N hydrochloric acid in methanol. In this way we have prepared both enantiomers of 3-fluoroalanine, which were used for peptide synthesis.

Papain has also been applied by us in the preparation of enantiomers of other fluorine-containing alanines as shown above. The optically active fluoroalanines have been obtained by this method in purities greater than 99% e.e. [13].

Enzymatic resolution of racemic mixtures of enantiomers of fluorine-containing amino acids provides an effective method for the preparation of the desired compounds. It is attractive because as a result of



resolution it is possible to obtain both enantiomers. For this reason, we also decided to examine the possibility of applying other enzymes for the resolution of enantiomeric mixtures of fluorine-containing amino acids.

Thus, for the resolution of a racemic mixture of (*R*,*S*)-fluorophenylalanines, we used methyl esters of these amino acids together with chymotrypsin, which hydrolyzes the methoxycarbonyl group. Chymotrypsin was found to hydrolyze the ester group of (*R*)-fluorophenylalanine at a pH value of 7.0 without participation of the (*S*)-enantiomer. By conducting the hydrolysis in acid medium, it is relatively easy to obtain the free amino acids. Individual enantiomers of phenylalanines containing fluorine atoms in the aromatic ring were obtained in high optical purity 98%–99% e.e. (HPLC data) [13].

As expected, the acid–base properties of fluorine-containing amino acids are appreciably different from those of the non-fluorinated amino acids. We have measured changes in the acidity and basicity of trifluoromethyl-containing amino acids. As shown by the data listed in Table 1, the greatest influence of the trifluoromethyl group is on the basicity of the amino groups [16].

We have also measured by potentiometric titration [17] the acid–base properties of carboxy and amino groups in the fluoroalanine series with increasing numbers of fluorine atoms (see Table 2).

From these data, it may be seen that the group most sensitive to fluorine atoms is the amino.

Table 1
Acid–base properties of amino acids CF_3–$(CH_2)_n$–CH(NH$_2$)–COOH

n	ΔpK_{COOH}	ΔpK_{NH_2}
3	0.17	0.13
2	0.23	0.81–0.89
1	0.54–0.75	1.32–1.66
0	0.56–1.13	3.53–4.41

Table 2
Acid–base properties of carboxy and amino groups in the fluoroalanine series

	pK_{COOH}	pK_{NH_2}
$FCH_2CH(NH_2)COOH$	2.4	9.8
$F_2CHCH(NH_2)COOH$	1.5	8.4
$F_3CCH(NH_2)COOH$	1.2	5.3

References

[1] J.T. Welch and S. Eswarakrishnan, *Fluorine in Bioorganic Chemistry*, Wiley, New York, 1991.
[2] V.P. Kukhar and V.A. Soloshonok, *Russ. Chem. Rev., 60* (1991) 850.
[3] V.P. Kukhar, Yu.L. Yagupolskii, I.I. Gerus and M.T. Kolycheva, *Russ. Chem. Rev., 60* (1991) 1050.
[4] V.P. Kukhar, *Izv. Akad. Nauk SSSR, Ser. Khim.,* (1990) 2290.
[5] C. Walsh, *Tetrahedron, 38* (1982) 871; V.P. Kukhar, Yu.L. Yagupolskii and V.A. Soloshonok, *Russ. Chem. Rev., 59* (1990) 89.
[6] M.T. Kolycheva, Yu.L. Yagupolskii, I.I. Gerus, L.F. Godunova and V.P. Kukhar, *Zh. Org. Khim., 25* (1989) 1306.
[7] L.F. Godunova, E.I. Karpeiskaya, E.S. Levitina, E.I. Klabunovksii, Yu.L. Yagupolskii and M.T. Kolycheva, *Izv. Akad. Nauk SSSR, Ser. Khim.,* (1987) 1359.
[8] L.F. Godunova, E.S. Levitina, E.I. Karpeiskaya, E.I. Klabunovskii, Yu.L. Yagupolskii and M.T. Kolycheva, *Izv. Akad. Nauk SSSR, Ser. Khim.,* (1989) 404.
[9] M.T. Kolycheva, I.I. Gerus, Yu.L. Yagupolskii, S.V. Galushko and V.P. Kukhar, *Zh. Org. Khim., 27* (1991) 781.
[10] I.I. Gerus, M.T. Kolycheva, Yu.L. Yagupolskii and V.P. Kukhar, *Zh. Org. Khim., 25* (1989) 2020.
[11] S.V. Galushko, M.V. Grigor'eva, M.T. Kolycheva, I.I. Gerus, Yu.L. Yagupolskii and V.P. Kukhar, *Bioorg. Khim., 16* (1990) 1924.
[12] M.T. Kolycheva, I.I. Gerus and V.P. Kukhar, *Amino Acids,* (1993) 99.
[13] M.T. Kolycheva, unpublished data.
[14] A.A. Kolomeitsev, Yu.L. Yagupolskii, I.I. Gerus and M.T. Kolycheva, *J. Fluorine Chem., 54* (1991) 319.
[15] I.I. Gerus, Yu.L. Yagupolskii, V.P. Kukhar, L.S. Boguslavskaya, N.N. Chuvatkin, A.V. Kartashov and Yu.V. Mitin, *Zh. Org. Khim., 27* (1991) 537.
[16] S.P. Kobzev, V.A. Soloshonok, S.V. Galushko, Yu.L. Yagupolskii and V.P. Kukhar, *Zh. Obshch. Khim., 59* (1989) 909.
[17] S.V. Galushko, I.P. Shishkina, I.I. Gerus and M.T. Kolycheva, *J. Chromatogr., 600* (1992) 33.