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Fluorine-containing aromatic aminoacids

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ABSTRACT. **Methods of preparing the following fluorine-containing aromatic aminoacids are reviewed: racemic and optically active phenylalanine and tyrosine derivatives with fluorine atoms or fluoroalkyl groups in the benzene ring or in the side chain. Possible bio-medical applications of these compounds are examined.**

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I. Introduction

The aromatic aminoacids—phenylalanine and tyrosine—are members of the essential aminoacids. They are constituents of important biologically active peptides, such as oxytocin, enkephalins, and kinins. The products obtained by metabolism of these aminoacids play a key role in processes which take place in the central nervous system and elsewhere in the living organism.

By replacement of atoms of hydrogen by fluorine in aminoacids, one can prepare compounds which are structurally very similar to the corresponding natural aminoacids since the size of the fluorine atom does not differ greatly from that of hydrogen. However, as the energy of the C–F bond is greater than that of the C–H bond, there is a significant change in the metabolism of derivatives containing fluorine. Furthermore, as a consequence of the electronegativity of the fluorine atom, there is a marked shift in electron density which has an affect on the physical chemical properties. With the development of organofluorine chemistry, new methods of selective fluorination have been designed and new fluorinating agents, as well as simple reactive fluorine-containing synthons, have become available. These have been usefully applied to the preparation of fluorine-containing aromatic aminoacids having atoms of fluorine or fluoroalkyl groups in various positions of the molecule. Interest in fluorine-containing phenylalanine and tyrosine is evident from the volume of publications in this area and from the applications of fluorine-containing aminoacids in biochemical studies and in medicine.

In 1970 a review was published on the synthesis of fluorine-containing aminoacids, including phenylalanine and tyrosine derivatives.[1] However, there is little mention of the biological activity of these compounds. A recent review covers the synthesis of aminoacids having a fluorine atom in the β-position of the hydrocarbon chain, including α-polyfluoromethyl derivatives.[2]

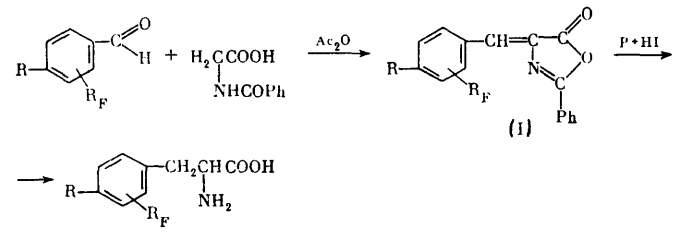
In the present literature survey, a systematic study has been made of synthetic routes to fluorine-containing derivatives of phenylalanine and tyrosine in both racemic and optically active form. The effect of fluorine substitution on biological activity has also been examined, and potential applications of these compounds in biochemical and medical research have been highlighted.

II. Synthesis of racemic fluorine-containing aromatic aminoacids

Various methods are available for preparing aromatic aminoacids with fluorine or fluoroalkyl substituents. These are based on either direct introduction of fluorine into the aminoacid molecule or syntheses from fluorine-containing residues and synthons. The latter methodology has been applied to the preparation of racemic fluorine-containing phenylalanine and tyrosine derivatives, which may be resolved into their enantiomeric forms by a variety of methods.

1. Methods based on the condensation of carbonyl compounds

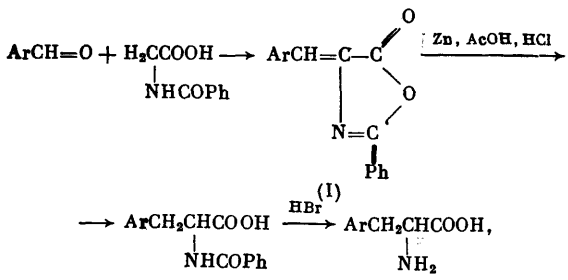
Phenylalanine and tyrosine derivatives with up to five fluorine atoms or trifluoromethyl groups in the benzene ring are usually prepared by the classical route from the corresponding fluorinated benzaldehydes and *N*-acetylglycine.[1] Normally, an oxazolone intermediate (I) is formed in high yield irrespective of the number of fluorine atoms in the aromatic nucleus. Subsequent reduction and hydrolysis (notably with a mixture of red phosphorus and HI) affords the desired fluorine-containing aromatic aminoacids.[1]



R = H, F, CF_3, MeO, OH; $R_F = F_n$, CF_3; $n = 1-5$

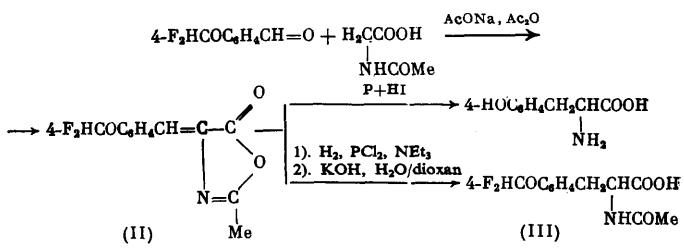
Fluorinated phenylalanine derivatives containing two, three or four atoms of fluorine at various positions of the benzene ring have also been prepared from the corresponding benzaldehydes via reduction of the intermediate oxazolones (I) under mild conditions (with Zn/AcOH). After acidic hydrolysis, ion-exchange resins have

been used to isolate the free aminoacids in higher yield and purity without contamination by inorganic salts.[3]

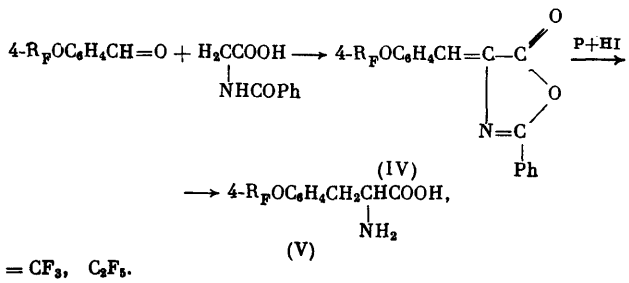


Ar = 2,4-$F_2C_6H_3$; 3,4-$F_2C_6H_3$; 3,5-$F_2C_6H_3$; 2,5-$F_2C_6H_3$; 2,6-$F_2C_6H_3$; 2,3,5,6-F_4C_6H; 2,3,4,6-F_4C_6H; 2,4,6-$F_3C_6H_2$; 3,5-Cl_2-2,4,6-F_3C_6.

4-Difluoromethoxybenzaldehyde, readily obtainable by treatment of 4-hydroxybenzaldehyde with chlorodifluoromethane, reacts with aceturic acid under the conditions of the Erlenmeyer azlactone synthesis to give the corresponding fluorine-containing unsaturated oxazolone (II). However, reduction of the latter with HI/P proceeds by cleavage of the difluoromethyl group to give tyrosine as the only isolable product. On the other hand, hydrogenation in the presence of Pd and NEt_3 affords methyl *N*-acetyl-4-difluoromethoxyphenylalanate which is converted to the *N*-acetyl derivative (III) on basic hydrolysis.[4]

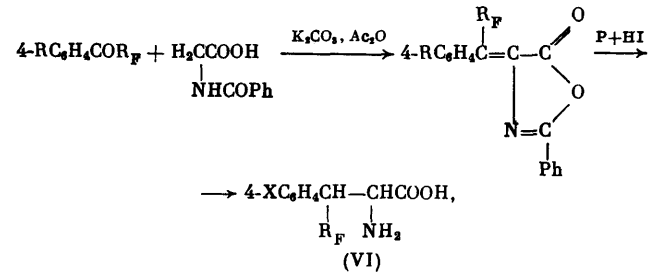


Benzaldehydes substituted by trifluoromethoxy and pentafluoroethoxy groups reacts with hippuric acid to give the corresponding fluorine-containing oxazolones (IV) which, in contrast to azalactone (II), is reduced by HI/P to the corresponding fluorine-containing aminoacids (V). Thus, perfluoroalkoxy groups remain intact under these conditions.[4]



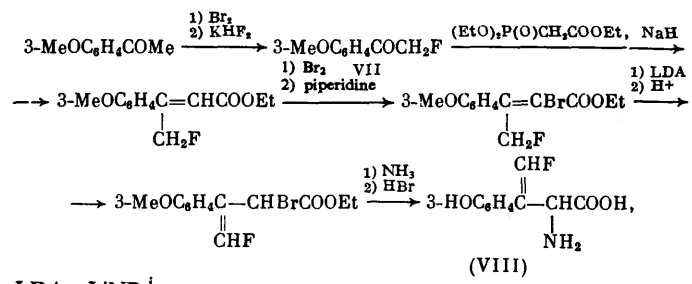
$R_F = CF_3$, C_2F_5.

The Erlenmeyer condensation of ω-polyfluoroacetophenones with hippuric acid affords the corresponding β-(polyfluoromethyl)-phenylalanines and -tyrosines. It should be pointed out that oxazolones are not formed under these conditions from acetophenone itself or from ω-monofluoroacetophenone.[5] Amino-acids (VI) have two chiral centres and are formed as a mixture of diastereoisomers.



R = H, OCH_3; X = H, OH; $R_F = CHF_2$, CF_3.

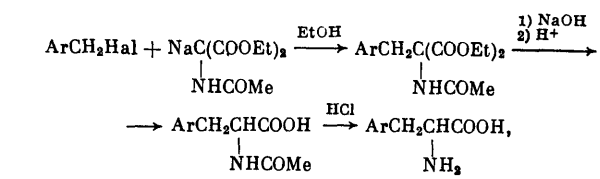
ω-Monofluoro-3-methoxyactophenone (VII) has been used as the key starting material for the synthesis of β-fluoromethylene-*m*-tryosine (VIII). Thus, Horner reaction with (VII) provides the corresponding substituted cinnamic acid ester which is converted by the route shown below to the *m*-tryosine (VIII)—an effective irreversible inhibitor of monoamineoxidase—in an overall yield of 32%.[6]



LDA – $LiNPr^i_2$.

2. Methods based on alkylation

The reaction of benzyl halides with *N*-acylaminomalonic acid esters has been generally applied to the synthesis of fluorine-containing aromatic aminoacids. Aminoacids may be obtained in high yield by this route. However, the sensitivity of the condensation to traces of moisture is a limitation which has a significant effect on the overall yield. In an earlier review,[1] there is reference to the synthesis of fluorine-containing phenylalanines and tryosines by reaction of the sodio-derivative of diethyl acetylaminomalonate with fluorine-containing benzyl halides.

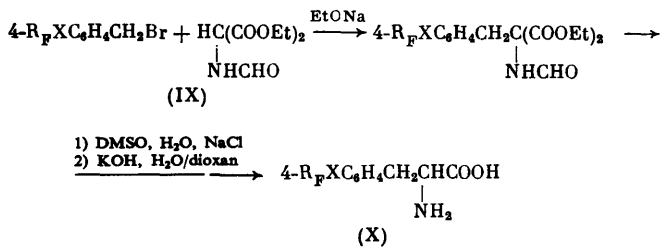


Hal = Cl, Br; Ar = 2-, 3- and 4-FC_6H_4, C_6H_4, C_6F_5, 4-HOC_6H_4, 3-$CF_3C_6H_4$.

As hydrolysis takes place in two steps, it is possible to isolate both the free aminoacid and its *N*-acetyl derivative. By alkylation with variously substituted fluoro- or trifluoromethyl-benzaldehydes,

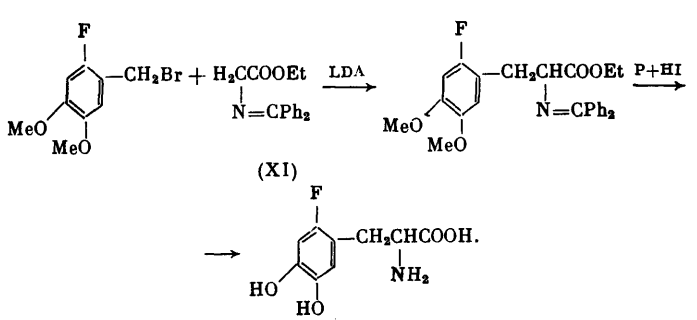
the following fluorine-containing aromatic aminoacids have been prepared: phenylalanine derivatives, $ArCH_2CH(NH_2)COOH$, where Ar = 2-FC_6H_4, 3-FC_6H_4, 4-FC_6H_4, 2,5-$F_2C_6H_3$, 3-$CF_3C_6H_4$, 2-Cl-5-$CF_3C_6H_3$, 4-Cl-3-$CF_3C_6H_3$;[7–9] tryosine analogues, where Ar = 2-F-4-$MeOC_6H_3$, 2-F-4-HOC_6H_3, 2,6-F_2-4-HOC_6H_2;[10–11] and the dihydroxyphenylalanine (DOPA) analogue where Ar = 2-F-3,4-$(HO)_2$-C_6H_2.[12]

Normally this route is based on diethyl acetylaminomalonate. However, it is possible to use other *N*-substituted malonates. Thus, condensation of 4-fluorobenzyl chloride with the corresponding phalimidomalonate, followed by hydrolysis in 5 *N* HCl at 100 °C affords 4-fluorophenylalanine in 50% yield (based on the starting benzyl halide).[13] By using the formamidomalonate (IX), removal of the protective group can be achieved under mild conditions so that fluorine-containing aromatic aminoacids with acid-labile substituents (e.g. R_FX = F_2HCO) may be prepared. Thus, treatment of fluorine-containing benzyl bromides with the sodio-derivative of (IX), followed by decarboxylation in aqueous DMSO with NaCl and basic hydrolysis, affords the corresponding polyfluoroalkoxy- and polyfluoromethylthio-phenylalanines (X) in 20–50% yield (based on the benzyl bromide).[4]

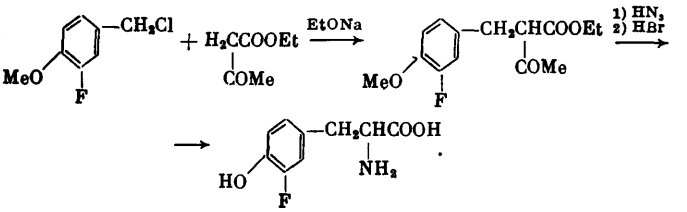


$R_FX = CHF_2O$, CHF_2S, CF_3S, CF_3CH_2O.

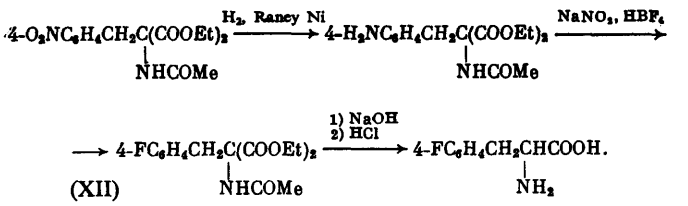
For the synthesis of fluorine-containing aminoacids from the corresponding benzyl halides, it is also possible to use other CH-acidic compounds, such as *n*-acylaminocyanoacetic acid esters,[1] in place of the acylaminomalonates. Thus, 6-fluoro-3,4-dihydroxyphenylalanine has been prepared from the glycinate Schiff base (XI), obtained from ethyl glycinate and benzophenone.[14] Generation of the carbanion in the initial deprotonation requires a strong base, e.g. LDA.



3-Fluorotryosine has been prepared by condensation of 3-fluoro-4-methoxybenzyl chloride with ethyl acetoacetate, followed by replacement of the acetyl group by an amino function with hydrazoic acid. A 63% yield has been recorded for this reaction.[15,16]



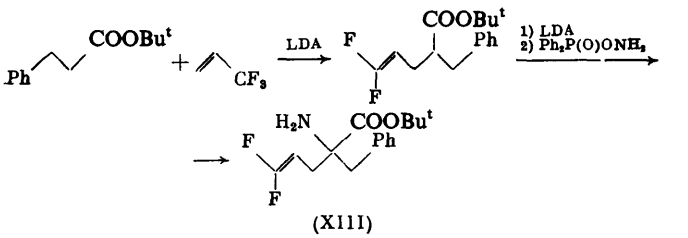
Introduction of the fluorine atom can take place during the course of the malonate-based route. Thus, after condensation of 4-nitrobenzyl chloride with the acetylaminomalonate, the nitro group may be reduced with H_2 over Raney nickel and fluorine introduced by the Schiemann reaction to give (XII).[17]



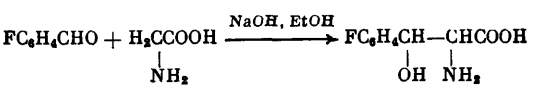
This method of introducing the fluorine atom *en route* is particularly useful for preparing phenylalanine labelled with the short-lived isotope ^{18}F ($\tau_{1/2}$ = 109 min).[18–20]

3. Other methods

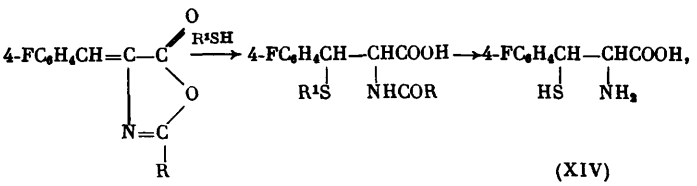
Recently, the phenylalanine derivative (XIII), having a γ,γ-difluoroallyl group in the α-position, was prepared from 3,3,3-trifluoropropylene, as shown below. *O*-Diphenylphosphinylhydroxylamine was used in the last step to introduce the amino group into the α-position relative to the carbonyl group.[21]



Treatment of 2- and 4-fluorobenzaldehyde with glycine in the presence of base affords the corresponding fluorine-containing β-phenylserine.[22,23]



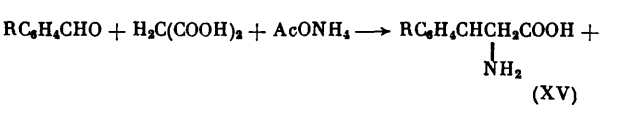
Fluorine-containing β-phenylthioserines have been prepared in the presence of base by reaction of mercaptans with the oxazolones obtained from α-acylamino-4-fluorocinnamic acids.[23] The free aminoacid (XIV) was formed on removal of the protecting groups.



(XIV)

R^1 = Et, $PhCH_2$.

A series of fluorine-containing aromatic aminoacids with an amino group in the β-position was prepared by the Rodionov reaction from benzaldehydes and malonic acid in the presence of ammonia. Thus, 4-fluorobenzaldehyde affords β-(4-fluorophenyl)-β-alanine in high yield.[23,24] Similarly, reaction with 3-fluoro-4-methoxybenzaldehyde, followed by treatment with HBr, affords β-(4-hydroxy-3-fluorophenyl)-β-alanaine.[24] β-[2- and 4-(Difluoromethoxy)phenyl]-β-alanines (XV) have also been prepared from the corresponding difluoromethoxybenzaldehydes.[25] The Rodionov reaction is accompanied by the formation of fluorine-containing cinnamic acids as by-product which may be removed by crystallisation.

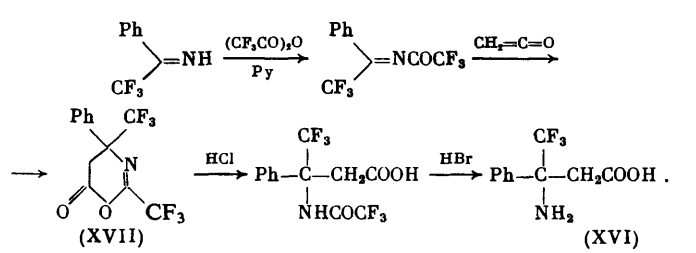


(XV)



R = 4-F, 4-$OCHF_2$, 2-$OCHF_2$.

The Rodionov reaction with ω,ω,ω-trifluoroacetophenone gives 4,4,4-trifluoro-3-phenyl-3-hydroxybutyric acid, rather than the expected β-trifluoromethyl-β-phenyl-β-alanine (XVI).[25] The latter has been obtained, however, by cyclisation of ω,ω,ω-trifluoroacetophenone *N*-trifluoroacetylimine with ketene, followed by hydrolysis of the resulting oxazinone (XVII).[26]



(XVII) (XVI)

Fluorine-containing aminoacids have also been prepared by direct fluorination of the parent aminoacids. A variety of reagents has been used for introducing a fluorine atom into the benzene ring of phenylalanine and tryosine. This route has also been applied to the preparation of homochiral fluorine-containing aminoacids, reaction proceeding as a rule with retention of chirality. The direct fluorination of (*S*)-phenylalanine and (*S*)-tyrosine will be discussed in the next section devoted to the synthesis of optically active aminoacids.

III. Synthesis of optically-active fluorine-containing aromatic aminoacids

Three routes are available for the preparation of optically-active aminoacids substituted by fluorine atoms or fluoroalkyl groups: resolution of racemic compounds into their enantiomers, for which various methods are reported; asymmetric synthesis; and fluorination of homochiral aminoacids with retention of chirality.

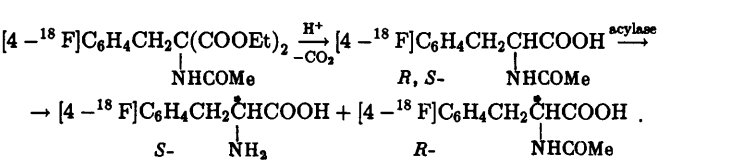
1. Optical resolution of fluorine-containing aromatic aminoacids

(*S*)- and (*R*)-Pentafluorophenylalanine have been isolated via recrystallisation of the diastereoisomeric salts formed on mixing *N*-benzoyl-(*R,S*)-pentafluorophenylalanine with brucine.[27] This method has been applied generally for the resolution of non-fluorinated aminoacids. However, it is laborious and low-yielding.

Chromatographic resolution of racemic aminoacids on chiral stationary phases is of much greater potential in this respect. This is exemplified by the optical resolution of 4-fluorophenylalanine.[28,29]

Enzyme-catalysed resolutions have been generally applied to the preparation of homochiral fluorine-containing derivatives of phenylalanine and tryosine. This route is based on the preferential enzyme-catalysed reaction of the *L*-enantiomer of a racemic mixture of a suitable aminoacid derivative. Thus, as long ago as 1946, the (*R*)- and (*S*)-enantiomers of 3-fluorotyrosine were isolated by papain-catalysed cleavage of racemic 3-fluorotyrosine anilide.[30] Similarly, papain-catalysed cleavage of racemic *N*-acetyl-(2-,3- and 4-fluorophenyl)alanine in the presence of phenylhydrazine afforded the (*S*)- and (*R*)-enantiomers of 2-, 3- and 4-fluorophenylalanine, respectively.[31] Mechanistically, the enzyme catalyses the formation of the corresponding *N*-acetyl-(*S*) fluorophenylalanine phenylhydrazide which is readily separated from the corresponding *N*-acetyl-(*R*)-fluorophenylalanine. The optically pure free aminoacids are obtained by subsequent hydrolysis of the resulting (*R*)- and (*S*)-fluorophenylalanine derivatives.

(*S*)-[4-^{18}F]-Fluorophenylalanine has been obtained by treatment of the racemic *N*-acetyl derivative with aminoacylase.[20] The use of aminoacylase is also recommended for the synthesis of homochiral aminoacids by the malonic ester synthesis, the enzyme substrates (*N*-acetyl derivatives) being formed without the additional requirement of an *N*-acylation step.

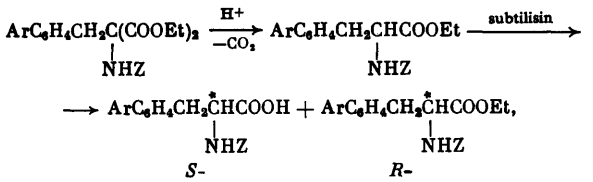


The enantiomers of pentafluorophenylalanine have been isolated via treatment of *N*-trifluoroacetyl-(*R,S*)-pentafluorophenylalanine with an acylase.[32]

α-Chymotrypsin has also been used for the enzymatic resolution of 4-fluorophenylalanine.[33] Thus, racemic 4-fluorophenylalanine is converted to the ethyl ester, which, on incubation with α-chymotrypsin affords (*S*)-4-fluorophenylalanine. The latter is

readily separated from the unreactive ethyl (*R*)-4-fluorophenylalanate. The same enzyme has been used for the resolution of methyl *N*-acetylpentafluorophenylalaninate.[34]

Subtilisin has been used[35] for the resolution of ethyl *N*-benzyloxycarbonyl-(*R,S*)-fluorophenylalaninates, obtained by the malonate route:



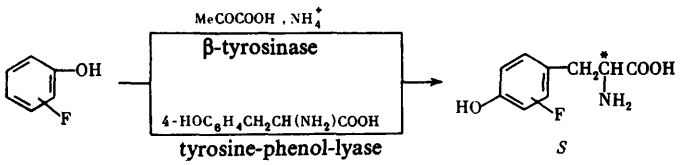
Ar = 3-FC$_6$H$_4$, 4-FC$_6$H$_4$, C$_6$F$_5$; Z = COOCH$_2$C$_6$H$_5$.

α-Chymotrypsin has also been used for the resolution of each of the three positional (*o*, *m*- and *p*-) isomers of methyl *N*-acetylfluorophenylalaninate and of *N*-acetyl-4-trifluoromethylphenylalaninate.[36] Carboxypeptidase A, however, is the preferred enzyme for the resolution of the *N*-trifluoroacetyl derivatives of these aminoacids.[36] All three positional isomers of *N*-benzyloxycarbonyl-(*S*)-fluorophenylalanine have been isolated with an enantiomeric excess (e.e.) of 90–97% by treatment of the appropriate racemic trifluoroethyl *N*-benzyloxycarbonylfluorophenylalanates with pig liver lipase.[37]

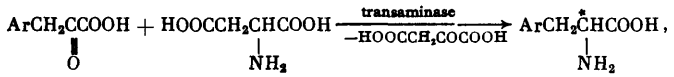
(*R*)-4-Fluorophenylalanine has been isolated by enzymatic resolution of *N*-benzoyl-(*R,S*)-4-fluorophenylalanine with an *Escherichia coli* culture.[38] All three of the positional isomers of fluorophenyl-alanine were resolved with lyophilised yeast, the (*R*)-enantiomers being isolated in 76–86% yield.[39]

2. Asymmetric synthesis

As well as being used for the optical resolution of racemic compounds, enzymes have also been applied to the synthesis of optically active fluorine-containing aminoacids. Thus, 2- and 3-fluoro-(*S*)-tyrosine have been prepared by treatment of the corresponding 3- and 2-fluorophenols with tyrosine-phenol lyase.[40,41]

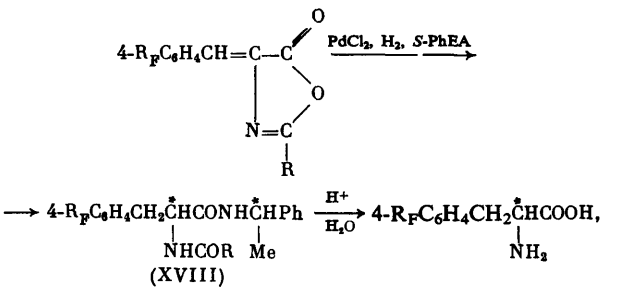


Fluorine-containing (*S*)-phenylalanines have also been synthesised. Thus, trifluoromethyl-, monofluoro-, and pentafluoro-(*S*)-phenylalanines have been prepared by action of transaminase on a mixture of aspartic acid and the appropriate fluorine-containing phenylpyruvic acid.[42]



Ar = FC$_6$H$_4$, CF$_3$C$_6$H$_4$, C$_6$F$_5$.

Reductive aminolysis has been used in the preparation of optically active *p*-fluoro- and *p*-difluoromethoxyphenylalanine.[43,44] Thus, reductive aminolysis of fluorine-containing oxazolones by hydrogenation over PdCl$_2$ in a variety of solvents containing (*S*)-(−)-α-phenylethylamine (PhEA) affords the corresponding *N*-acyl-(4-fluoro- or 4-difluoromethoxy-phenyl)alanine α-phenylethylamide (XVIII), predominantly in the (*S,S*)-configuration, with a diastereoisomeric excess (d.e.) up to 47%.

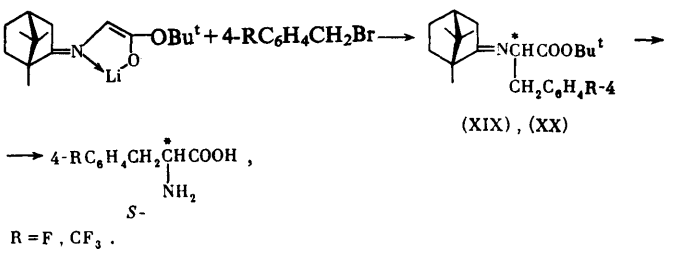


(XVIII)

R$_F$ = F, OCHF$_2$; R = Me, Ph.

Crystallisation of the *p*-fluorophenylalanine amides affords the optically pure (*S,S*)-diastereoisomers, hydrolysis of which in 6 *N* HCl yields enantiomerically pure 4-fluoro-(*S*)-phenylalanine.[43] Attempts to cleave the amide bond in *p*-difluoromethoxyphenylalanine analogues leads to removal of the difluoromethyl group to give (*S*)-tyrosine.[44]

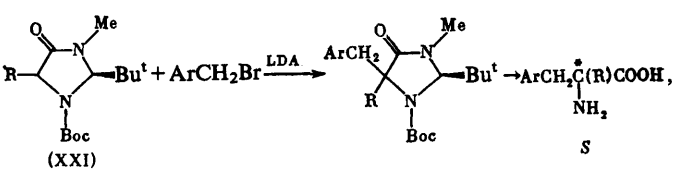
Fluorine-containing aromatic aminoacids have also been synthesised via alkylation with asymmetric induction. This is exemplified by the alkylation of the Schiff base derived from *tert*-butyl glycinate enolate and (1*R*)-(+)-camphor with fluorine-subtituted benzyl bromides.[45] Reaction proceeds with asymmetric induction to give the corresponding 4-fluoro- and 4-trifluoromethyl-phenylalanate Schiff bases (XIX and XX) with a d.e. of 98 and 76%, respectively. Hydrolysis of (XIX) and (XX) affords the corresponding (*S*)-aminoacid.



(XIX), (XX)

R = F, CF$_3$.

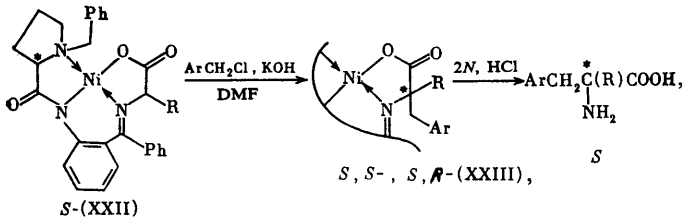
Stereoselective alkylation of chiral 4-imidazolidones (XXI) with pentafluorobenzyl or *p*-fluorobenzyl bromide also provides fluorine-containing aromatic aminoacids.[46,47] Thus, optically active α-methyl-4-fluorophenylalanine is obtained by alkylation of the diastereoisomeric alanine derivative (XXI), R = Me, with 4-fluoro-benzyl chloride, followed by hydrolysis.[48]



(XXI)

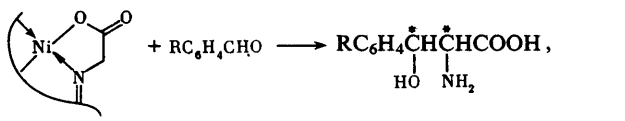
R = H, Me; Ar = 4-FC$_6$H$_4$, C$_6$F$_5$; Boc = COOCMe$_3$.

Asymmetric synthesis of (*S*)-fluorophenylalanines has been achieved from a homochiral nickel Schiff base complex prepared from (*S*)-2-[*N*-(*N'*-benzylprolyl)-amino]benzophenone and glycine.[49] Thus, complex (XXII) is alkylated with fluorobenzyl chlorides in DMF containing KOH to give a 95:5 mixture of the diastereoisomeric complexes, (*S,S*)- and (*S,R*)-(XXIII). The major isomer, (*S,S*)-(XXIII), may be isolated in optically pure form, and converted to the corresponding (*S*)-fluorophenylalanine (optical purity >99%) by treatment with HCl. The chiral prolyl ligand is also liberated and may be re-used.



R = H, Me; Ar = 2-FC_6H_4, 3-FC_6H_4, 4-FC_6H_4.

A number of diastereoisomeric β-phenylserines may also be prepared by reaction of the chiral complex, (*S*)-(XXII), with fluorine-substituted benzaldehydes.[50]



R = 2-F, 4-F 2-$OCHF_2$, 4-$OCHF_2$, 4-OCF_3, 3-CF_3.

3. Direct fluorination of enantiomeric aminoacids

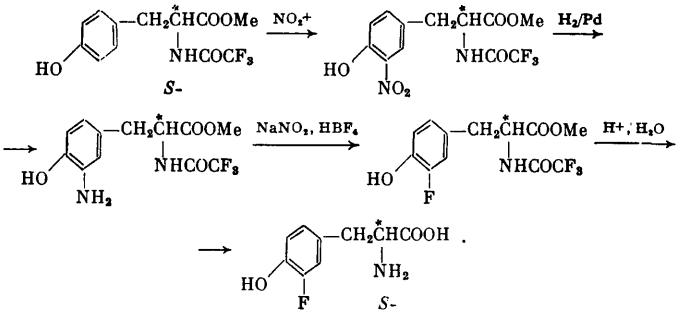
One of the most viable routes to enantiomeric fluorine-containing aminoacids involves introduction of the fluorine atom into the aminoacid molecule with retention of optical activity. Such methodology avoids multistep sequences and is particularly useful for preparing [^{18}F]-fluorinated aminoacids. Elemental fluorine can be the fluorinating agent as well as such auxiliaries as acetyl hypofluorite and XeF_2.

Thus, (*S*)-3-fluorotyrosine has been prepared via fluorination of *O*-methyl-(*S*)-tyrosine with acetyl hypofluorite.[51]



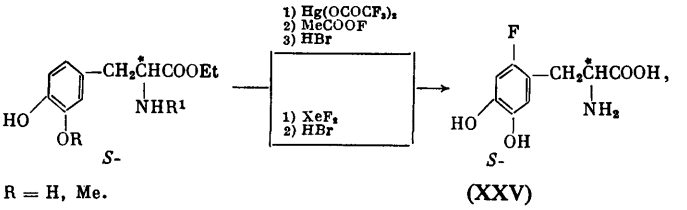
Enantiomeric (*S*)-monofluorophenylalanine and (*S*)-[3-^{18}F]-fluorotyrosine labelled with ^{18}F have also been obtained by this route.[52] Elemental fluorine has also been used for the ring-fluorination of aromatic aminoacids. Thus, treatment of a solution of tyrosine in hydrofluoric acid with N_2-diluted gaseous fluorine affords 3-fluorotyrosine in 46% yield, while fluorination of *O,N*-diacetyltyrosine in trifluoroacetic acid gives a 2:1 mixture of 2- and 3-fluorotyrosine.[53] (*S*)-Fluorophenylalanines with an ^{18}F label in the benzene ring were also prepared by this route;[54] the resulting mixture of labelled (*S*)-2-, (*S*)-3- and (*S*)-4-fluorophenylalanaine was separated by HPLC.

In order to avoid the formation of isomeric fluorine-containing aminoacids, methods have been devised for the regioselective introduction of fluorine atoms into the aromatic nucleus. This is exemplified by the synthesis of 3-fluoro- and 3,5-difluoro-(*S*)-tyrosine.[55]



It is based on the nitration of a protected (*S*)-tyrosine derivative, followed by reduction of the nitro group and introduction of the fluorine atom by the Schiemann reaction. Reaction proceeds with retention of chirality at all stages of the conversion, and provides (*S*)-3-fluorotyrosine in 21% yield.

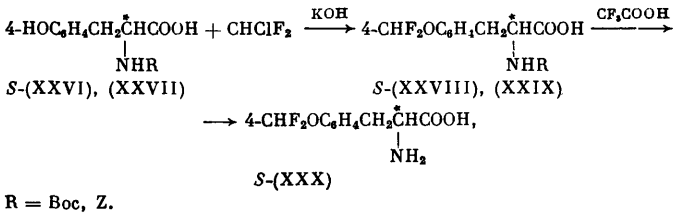
A procedure for introducing a fluorine atom regiospecifically at the 6 position of 3,4-dihydroxyphenylalanine has also been reported.[56] Thus, treatment of protected (*S*)- or (*R*)-3,4-dihydroxyphenylalanine derivatives with mercuric trifluoroacetate affords exclusively the corresponding 6-mercuri-derivatives, which are converted to the respective homochiral 6-fluoro-3,4-dihydroxyphenylalanines (XXV) in 20% yield via fluorination with acetyl hypofluorite. Homochiral (XXV) was also obtained via direct fluorination of a 3-methoxy-4-hydroxyphenylalanine derivative with xenon difluoride;[57] the yield of product after removal of the protective groups, was 25% (based on XeF_2). Both reactions proceed with retention of chirality.



R = H, Me. (XXV)

The procedure for polyfluoroalkylation of phenols[58] has recently been applied to the synthesis of polyfluoroalkylated (*S*)-tyrosine derivatives.[58] Thus, treatment of *N*-Boc-(*S*)-tyrosine (XXVI) or *N*-*Z*-(*S*)-tyrosine (XXVII) with chlorodifluoromethane in the presence of base affords the corresponding optically active *N*-protected *O*-difluoromethyltyrosine derivatives (XXVIII and

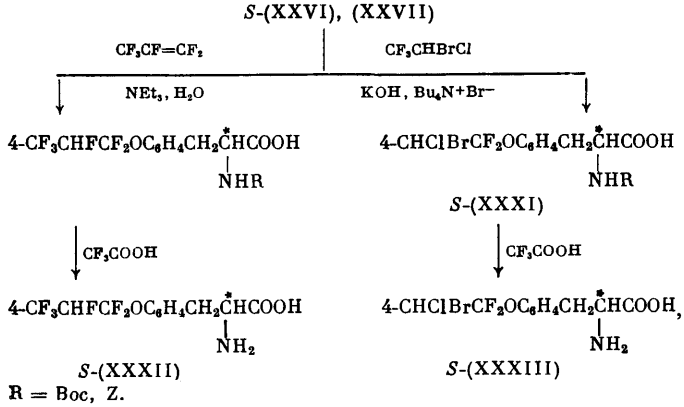
XXIX), which may be used in peptide synthesis as the carboxyl component.



R = Boc, Z.

The free aminoacid (XXX) was isolated by action of trifluoroacetic acid on the Boc-derivative (XXVIII). With the aid of ligand-exchange HPLC on a chiral stationary phase it was shown that the difluoromethylation proceeds without racemisation, and that the optical purity of (*S*)-(XXX) was >99.5%.

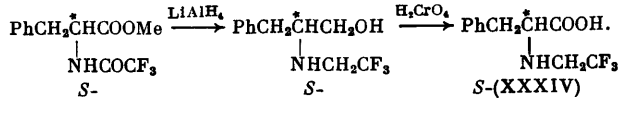
In a similar way,[58] *bis*(difluoromethylation) of *N*-Boc-(*R,S*)-3,4-dihydroxyphenylalanine affords 3,4-*bis*(difluoromethoxy)-(*R,S*)-phenylalanine in 14% yield. The interaction of (*S*)-tyrosine derivatives, (XXVI and XXVII), with hexafluoropropylene in aqueous triethylamine at 70 °C provides the corresponding 1,1,2,3,3,3-hexafluoropropoxy-(*S*)-aminoacids. On the other hand, the 4-polyhalogenoethoxy derivative of *N*-Boc-(*S*)-phenylalanine (XXXI) has been prepared by treatment of *N*-Boc-(*S*)-tyrosine (XXVI) with fluorothane, $CF_3CHBrCl$ (a commercially available inhalant-type anaesthetic), in 50% aqueous KOH containing $Bu_4N^+Br^-$ as phase transfer catalyst.



R = Boc, Z.

The corresponding unprotected polyfluoroalkoxy derivatives of (*S*)-phenylalanine (XXXII and XXXIII) were obtained by cleavage of the *tert*-butoxycarbonyl group in trifluoroacetic acid at 20 °C. The e.e. exceeded 95%, as was demonstrated by ^1H NMR spectroscopy with the aid of the chiral lanthanide shift reagent, Eu(hfc)$_3$. Thus, it has been confirmed that *O*-polyfluoroalkylation of *N*-protected (*S*)-tyrosine derivatives proceeds without racemisation.

Optically active methyl *N*-trifluoroacetyl-(*S*)-phenyalanate has been converted to *N*-trifluoroethyl-(*S*)-phenylalanine (XXXIV) by reduction with $LiAlH_4$, followed by oxidation of the resulting optically active aminoalcohol with Jones' reagent.[59]



IV. Possible fields of application

The replacement of hydrogen by fluorine in the aminoacid molecule does not introduce any steric hindrance since the van der Waal's radius of the atom fluorine is approximately the same as that of hydrogen. As a consequence, living organisms cannot normally distinguish fluorinated aminoacids from natural ones so that the former may be readily incorporated. This phenomenon is called the "masking effect", and is the rationale for using fluorinated aminoacids in biochemical and medical research into the study of metabolic pathways and the mechanism of action of various enzyme systems. In this respect, ^{19}F NMR spectrosocpic analyses and radioactive labelling (notably with the short-lived ^{18}F isotope) have been fruitfully applied in this field of research.

In studying the activity of phenylalanine-ammonia-lyase, it has been shown that deamination of (*S*)-4-fluorophenylalanine proceeds at half the rate of the same reaction with (*S*)-phenylalanine.[60] The substrate specifically of such enzymes as phenylalanine-*t*-RNA-ligase,[61] aspartate-transaminase,[62] chorismate-mutase,[63] lacto-peroxidase,[64] and tyrosine-phenol-lyase[65] has been studied by using 2-fluoro-, 4-fluoro- and 3,4-difluoro-phenylalanine as well as 3-fluorotyrosine. A study has also been made of the effect of molecular geometry and electronic charge distribution on the interaction of aminoacid oxidases with substrates and inhibitors;[6] a determination has been made of the correlation between the rate of degradation of aromatic aminoacids and the Hammett constant for substituents in the aromatic ring. 3-Fluoro- and 4-fluoro-phenylalanine were substrates for this study.

A study of the deactivation of leucocytic elastase by aromatic aminoacid derivatives has shown that deactivation is enhanced if there is a trifluoromethyl group in the *m*-position of the aromatic ring of phenylalanine. This is a consequence of an increase in the hydrophobicity of the molecule.[67]

By applying reverse-phase HPLC, a quantitative assessment has been made of the lipophilicity of phenylalanines containing fluoro-groups in the *p*-position. The change in free energy of sorption was measured as a function of the number of fluorine atoms in the molecule. The increase in hydrophobicity (π_x) for a series of fluorine-containing groups follows the order shown below.[58]

R_F	F	$OCHF_2$	$OCF_2CHClBr$	OCH_2CF_3	$SCHF_2$	OCF_3	SCF_3	OC_2F_5	OCF_2CHFCF_2
π_x	0.14	0.57	0.69	0.93	0.95	1.06	1.43	1.80	1.90

The initial study of 4-fluorophenylalanine metabolism showed that it was converted to tyrosine.[68] However, more recent research has revealed that the formation of tyrosine from 4-fluorophenylalanine proceeds six times more slowly than from phenylalanine.[69] This is explained by the higher C–F bond energy (116 Kcal/mol) relative to the C–H bond (99 Kcal/mol).

4-Fluorophenylalanine is a competitive antagonist of phenyl-alanine.[70] It also possesses fungicidal properties towards a number of pathogenic fungi which infect cucumbers and potatoes.[71]

A study of the physiological activity of 3-fluorotyrosine in mice has shown that this amino-acid, which participates in the normal metabolism of tyrosine, is converted to monofluoroacetic acid. The latter blocks the Krebs cycle, thereby inducing convulsions and death.[72]

A series of fluorinated derivatives of phenylalanine and tyrosine have been found to inhibit the growth of *Neurospora crassa*. 3-Fluorotyrosine is the most active inhibitor in this respect.[73] 2-Flurotyrosine also inhibits the growth of *Escherichia coli*,

Streptococcus faecalis, and other bacteria,[74] while 4-fluorophenylalanine inhibits the development of malignant tumours.[75–78]

In studying the substrate specificity of monoamine oxidase (MAO B)—an enzyme which plays a key role in the formation of neurotransmitters in the central nervous system (CNS)—it has been shown that β-fluoromethylene-*m*-tyrosine (VIII) is an effective irreversible inhibitor of MAO B in the presence of aromatic amino-acid decarboxylase.[79–81] β-Polyfluoromethyl-substituted phenylalanines and tyrosines (VI), which are structurally similar to aminoacid (VIII), have a sedative effect on the CNS.[5]

In studying the effect of introducing fluorine and trifluoromethyl groups at various positions of the phenylalanine and tyrosine ring on the opioid activity of enkephalin, it was demonstrated that substitution of phenylalanine by 4-fluorophenylalanine leads to a significant increase in enkephalin activity; however, introduction of a trifluoromethyl group at the *para*-position of phenylalanine, or substitution of tyrosine by 4-fluorophenylalanine, reduces the activity of the peptide.[82]

The decapeptide, P-Glu-*D*-*p*-F-Phe-Trp-Ser-Tyr-*D*-Ala-Leu-Arg-Pro-Gly-NH_2, having a (*R*)-4-fluorophenylalanine residue in the 2 position displays anti-ovulating activity in mammals,[83] while tripepetides with a (*S*)-4-fluorophenylalanine residue show analgesic activity.[84] Peptides containing fluorinated aromatic aminoacids, especially fluorophenylalanine, possess anti-tumour activity,[85] and have been used as medicaments.[86]

A large number of bradykinin and oxytocin analogues have been synthesised for studies of structure-activity relationships and the mechanism of action of these two peptides. Particular emphasis has been placed on susbtituting phenylalanine by 4-fluorophenylalanine (in oxytocin)[87] and by 3-trifluoromethylphenylalanine (in bradykinin).[88]

As fluorophenylalanine and fluorotyrosine derivatives participate in metabolic processes, they may be incorporated in proteins.[89,90] Thus, on administration of 4-fluorophenylalanine to rabbits, it was shown that this aminoacid is incorporated in the protein of muscles, the liver, and the blood (by replacing phenylalanine). Being incorporated in enzymes, 4-fluorophenylalanine may influence their activity, or have no effect at all, depending on the level of 4-fluorophenylalanine incorporation.[91]

[^{18}F] containing aromatic aminoacids are used for studying the metabolism of fluorinated aminoacids and enzymatic reactions.[92] On studying the distribution of 4-fluorophenylalanine-^{18}F in pig organs, it has been shown that most of the labelled aminoacid is incorporated in the pancreas.[93] Clincially, 4-fluorophenylalanine-^{18}F may be used for scanning this organ.[94,95]

A study of the metabolism of (*S*)-6-[^{18}F]-3,4-dihydroxyphenylalanine has shown that this compound is metabolised more quickly than ^{14}C-labelled 3,4-dihydroxyphenylalanine, which has shed light on the pathways by which this aminoacid is metabolised.[96]

^{19}F NMR spectroscopy has found widespread application in biological studies.[97] This is exemplified by a study of bacteriorodopsin containing 3-fluorotyrosine,[98] of the mechanism of transamination with 4-fluorophenylalanine in the presence of pyridoxal phosphate-dependent enzymes,[99] and of the enzymatic hydrolysis of 3-fluorotyrosine phosphate.[100]

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From this analysis of published data, it is clear that fluorine-containing aromatic aminoacids have a valuable application in the following areas: specific modification of physiologically active peptides; the study of the structure and mechanism of action of complex biologically active molecules with the aid of ^{19}F NMR; and the preparation of effective regulators of bio-systems. Thus, the development of new methods of synthesis of fluorine-containing aromatic aminoacids, especially optically active derivatives, is both timely and potentially fruitful.

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