Advances in the Synthesis of Bioactive Unnatural Amino Acids and Peptides

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Abstract: The key role of proteins and amino acids in the structure and function of living matter has stimulated extensive studies. Modified amino acids with enhanced biological activity, proteolitic stability and bioavailability are of increasing interest in protein design and engineering as drug candidates. In the last few years, several efforts have been devoted to the synthesis of amino acids having unusual side chains and unnatural chirality, commonly referred to as "nonproteinogenic" or "unnatural" amino acids, even though some of them can be isolated from natural sources. In this review we describe recent advances in the amino acid side-chain transformations and backbone modifications by oxidative and fluorination procedures.

Keywords: Unnatural amino acids, peptidomimetics, biological activity, synthesis, structural preperties.

INTRODUCTION

Unnatural or nonproteinogenic amino acids are characterized by several biological activities such as antimicrobial [1], antiviral [2], metal chelating properties [3], thrombin, trypsin and factor VIIa inhibitory activity [4]. Moreover, they show a highly potent and selective agonist activity for group II metabotropic glutamate receptors [5] and hypolipidemic activity [6]. Several efforts have been devoted to design novel synthetic procedures for the preparation of these derivatives. Due to the abundance of examples, we have limited ourselves to analyze the transformations of amino acids and peptides in the side-chain residue and in the backbone structure focusing our attention on the oxidative and fluorination procedures published mainly in the last ten years, with a particular emphasis on the synthesis of biologically active compounds. For sake of completeness, recent reviews are suggested for otherwise relevant topics in the field of unnatural amino acids preparation, such as the synthesis of nonproteinogenic amino acids in enantiomerically pure form [7], biomimetic organometallic amino acids [8], catalytic asymmetric Mannich reaction [9], developments in the application of organometallic chemistry for amino acid synthesis [10], cycloaddition reactions [11], biocatalysis [12], foldamers study [13], α,α-disubstituted amino acids and peptides [14], cyclic peptides [15] and unsaturated amino acids and peptides [16].

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SIDE-CHAIN RESIDUE AND BACKBONE OXIDATIVE TRANSFORMATIONS

Procedures Based on Ru^{VIII} Species

The Ru^{VIII} species generated in situ from catalytic $RuCl_3 \cdot 3H_2O$ and excess of sodium periodate have been largely used for the oxidative side-chain transformation of coded amino acids. For example, N^{α}-benzyloxycarbonyl histidine methyl ester (N^{α}-Z-HisOMe) **1** and N^{α}-benzoylhistidine methyl ester (N^{α}-Bz-HisOMe) **2** are selectively oxidized by Ru^{VIII} species in $H_2O/CH_3CN/CCl_4$ mixture at the histidine ring to yield non coded amino acids side chain having biological interest, such as N^{α}-benzyloxycarbonyl-N^{ω}-formyl asparagines methyl ester **3** (22%), *N*-benzyloxycarbonyl aspartic acid-α-methyl ester **4** (25%), N^{α}-benzoyl-N^{ω}-formyl asparagine methyl ester **5** (31%) and *N*-benzoyl aspartic acid-α-methyl ester **6** (34%), respectively (Scheme **1**) [17]. It is interesting to note that N^{ω}-carbamoyl asparagine, arising from **3**, is an analogue of insecticidal non-coded plant amino acid *L*-alibizzine (N^{β} – carbamoyl-β-amino alanine), a competitive antagonist of asparagines [18].

Compounds **3**-**6** are formed by a Ru^{VIII} mediated scission reaction at the imidazole 4,5 double bond, followed by water addition and further rearrangement of the molecule. In principle, this oxidation, followed by the removal of the protective groups, is an efficient synthetic protocol to selectively transform the histidinyl residue into asparagine or aspartic acid moieties. In a similar way, tryptophan is transformed to aspartic acid by Ru^{VIII} species. Thus, treatment of *N*-benzoyl tryptophan methyl ester **7** or *N*-benzyloxycarbonyl tryptophan methyl ester **8** with 2.2 mol % of Ru^{VIII} reagent and excess of sodium periodate afforded the corresponding aspartic acid derivatives **9**-**10** as the main reaction products, besides the partially degraded side-chain derivative **11** as a by-product (Scheme **2**) [19]. Most

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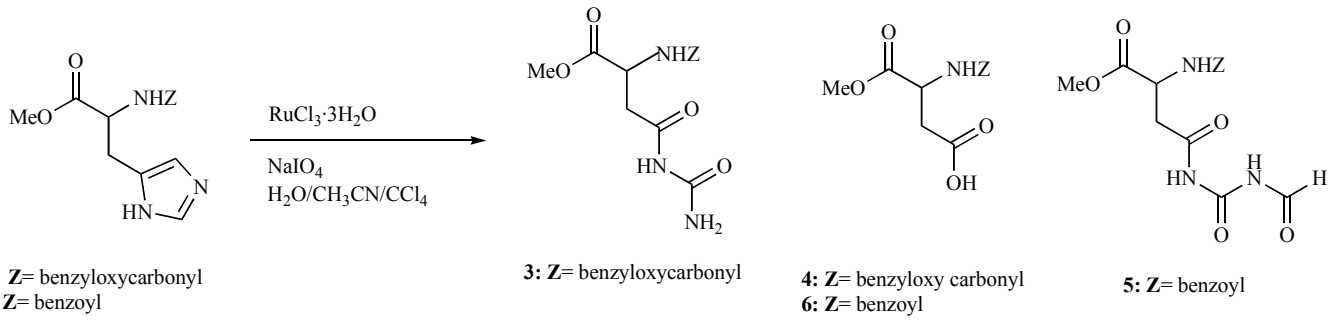


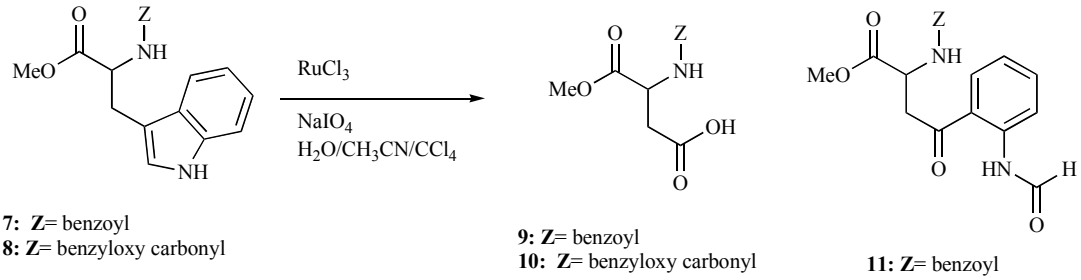
1: **Z**= benzyloxycarbonyl
2: **Z**= benzoyl

3: Z= benzyloxycarbonyl

4: Z= benzyloxy carbonyl
6: Z= benzoyl

5: Z= benzoyl

Scheme 1.



7: Z= benzoyl
8: Z= benzyloxy carbonyl

9: Z= benzoyl
10: Z= benzyloxy carbonyl

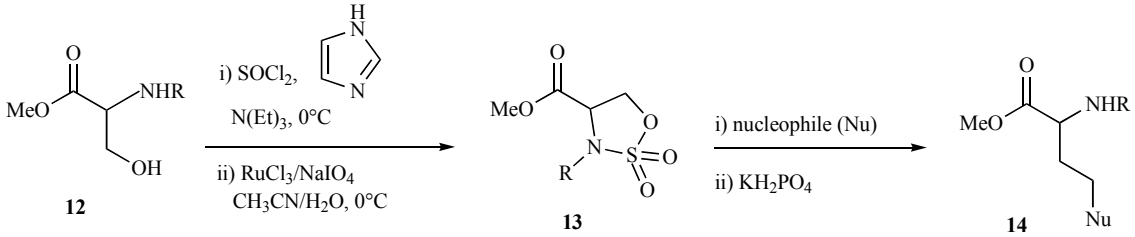
11: Z= benzoyl

Scheme 2.

probably, the transformation proceeded in four stages including the initial oxidative ring-opening of the pyrazole ring with formation of **11** as intermediate, followed by removal of the formyl moiety, aromatic degradation and oxidative decarboxylation. Tryptophan-containing peptides were also oxidized to corresponding aspartic acid derivatives.

The reaction showed a high selectivity in the presence of Phe residues which are known to undergo oxidation to Asp with Ru^{VIII} [20]. Ru^{VIII} species have been also used to introduce nucleophiles, including enolates of β-dicarbonyl compounds, in the side-chain of serine derivatives. In particular, the reactive cyclic sulfamidate intermediate **13** was prepared (in two steps) by reaction of serine methyl ester **12** with $SOCl_2$ in the presence of imidazole, followed by oxidative ring-closure with $RuCl_3$ and sodium periodate. Compound **13** was then subjected to a series of nucleophilic substitutions to yield a large panel of non-coded amino acid derivatives **14** (Scheme **3**) [21].

A similar procedure applied to homoserine-derived cyclic sulfamidate afforded a variety of γ-substituted α-amino acid analogues, including a set of enantiopure α, γ-diamino acid analogues [22]. Recently, an efficient method for the synthesis of enantiopure β- and δ- amino acids by ruthenium-catalyzed oxidative degradation of phenyl groups on the side-chain of α-amino acids was reported [23]. Oxidative modifications of the peptide backbone can also be performed with Ru^{VIII} species. For example, *N*,*C*- protected peptides terminating in Ser and Thr residues are subjected to oxidative scission of the C^{α}-C side chain by treatment with $RuCl_3$ and sodium periodate to yield corresponding terminal amides [24]. This transformation is biomimetic of the terminal amidation process in the cell associated with the formation of pituitary hormones from their Gly extended precursors [25]. As suggested in Scheme **4**, the oxidation of the dipeptide Bz-Ala-Ser/Thr-OMe **15** proceeds through initial coordination of Ru^{VIII} species on Ser/Thr side-chain with the formation of cyclic or alicyclic intermediate **A**, that can undergo the oxidative C^{α}-C scission to yield the carbinolamide intermediate **B**. This intermediate, in turn, may also undergo further oxidation to oxalamido derivatives **16** and to terminal amides **17** by hydrolytic cleavage [26]. The procedure showed a high selectivity because Gly, Ala, Leu, Asn, Asp, Glu, Phe, Arg and Val residues were not affected during the oxidation. When the Ser/Thr residues are placed in a peptide sequence either at the nonterminal or at *N*-terminal position, the α-ketoamide (NH-CO-CO) residue

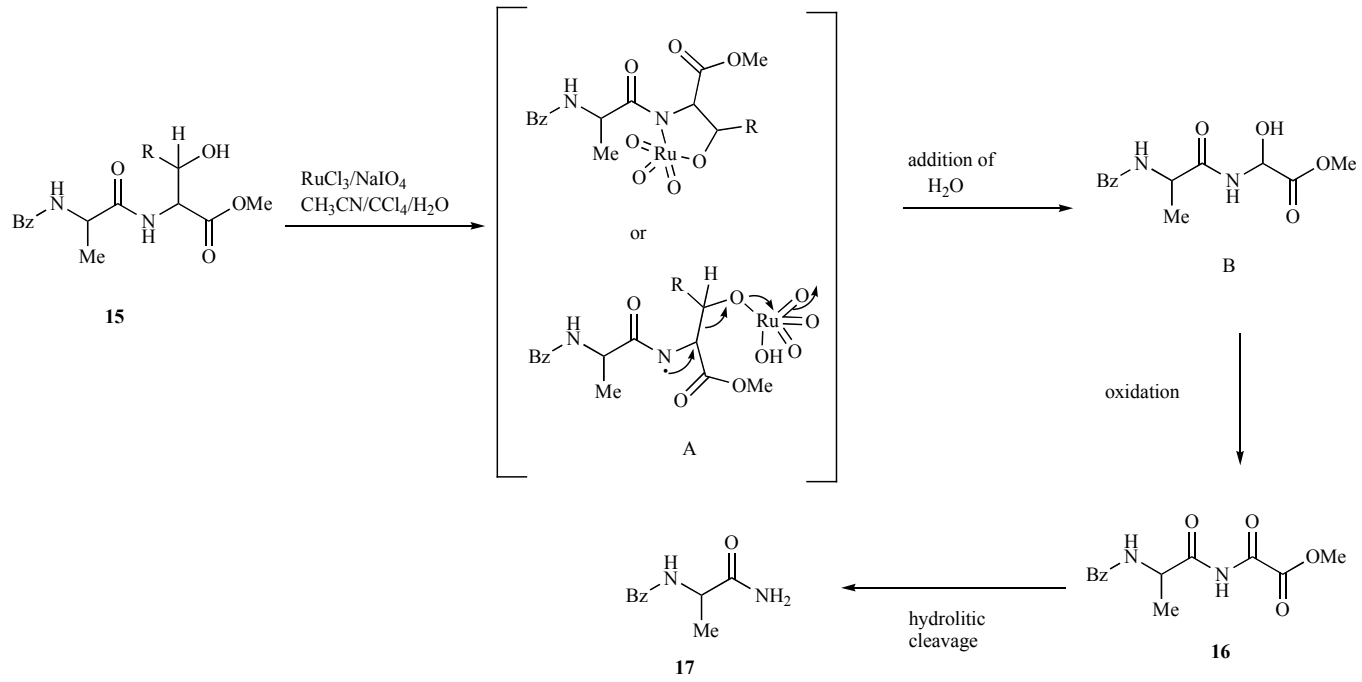


12

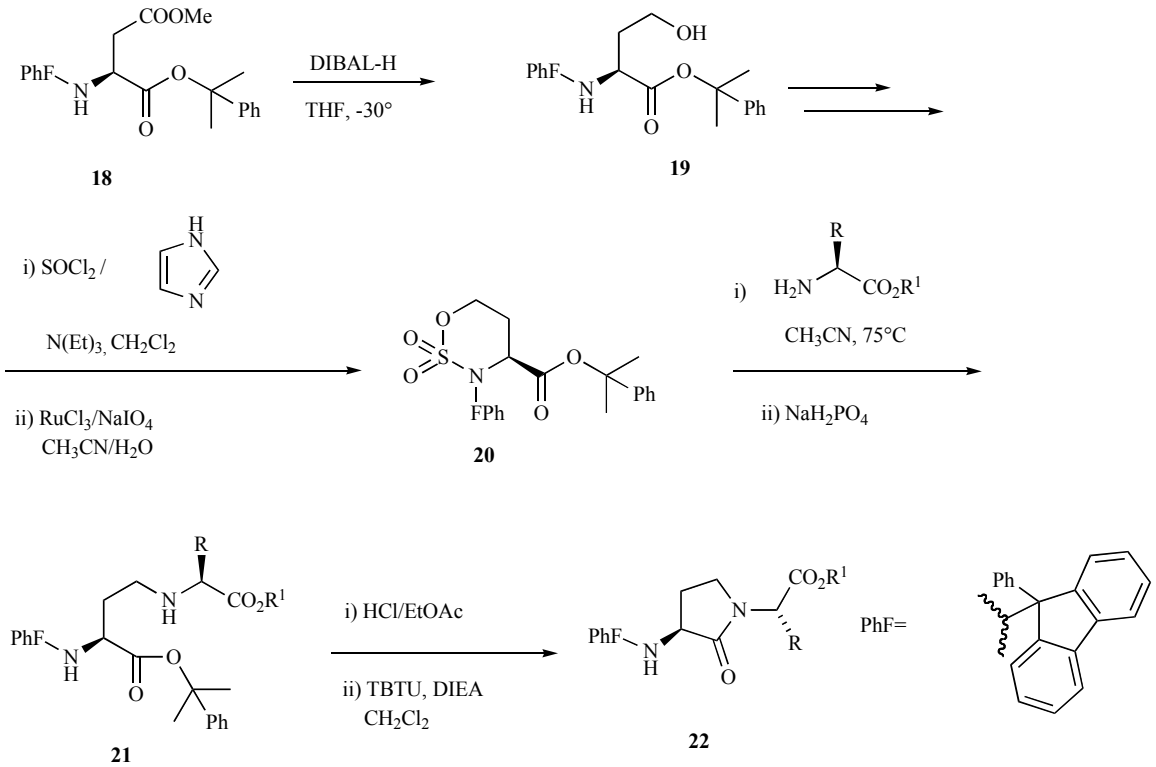
13

14

Scheme 3.



Scheme 4.



Scheme 5.

in place of the oxidized amino acid was obtained (Scheme **4**).

Another interesting example of the use of Ru^{VIII} species for the oxidative modification of the peptide backbone is the introduction of an α-amino lactam ("Freidinger lactam") moiety into the peptide chain. In fact, peptides containing α-amino lactams embedded in their sequence show improved biological activities [27]. As a general procedure, protected α-amino, γ-lactam-bridged dipeptides have been prepared starting from *N*-[9-(9-phenylfluorenyl)]-*L*-aspartic acid, α-cumyl-β-methyl diester **18** [28] (Scheme **5**). After selective reduction of **18** with diisobutylaluminum hydride (DIBAL-

H), homoserine **19** was successively treated with $SOCl_2$/imidazole and $RuCl_3/NaIO_4$ to yield the sulfamidate derivative **20**. Finally, a two-step process including cleavage of the cumyl ester and activation of the carboxylate moiety afforded desired lactam-bridged dipeptides **22**. This procedure was also applied for the synthesis of a γ-lactam analogue of the dopamine receptor modulator Pro-Leu-Gly-NH_2 (PLG).

Procedures Based on Generation of Reactive Oxygen Centered Free Radical Species

Generation of oxygen centered reactive free radical species represent an alternative pathway for the oxidative transformation of amino acids and peptides either on the side-chain residues and peptide backbone. In this context, the activation of hydrogen peroxide (H_2O_2) by copper ions received a great interest due to the correlation between the oxidative stress and different cell disorders [29]. Cu(II) ions in the presence of H_2O_2 are reduced to Cu(I) ions with the generation of the superoxide anion (Equation 1, line A). In a cyclic way, Cu(I) reacts with H_2O_2 to yield the highly reactive hydroxyl radical ($HO^{\cdot}$) while superoxide in acidic media dismutates to H_2O_2 and O_2 (Equation 1, line B) [30].

A) $Cu(II) + H_2O_2 \longrightarrow Cu(I) + O_2^{-\cdot}$

B) $Cu(I) + H_2O_2 \longrightarrow Cu(II) + HO^{\cdot} + HO^-$

Equation 1.

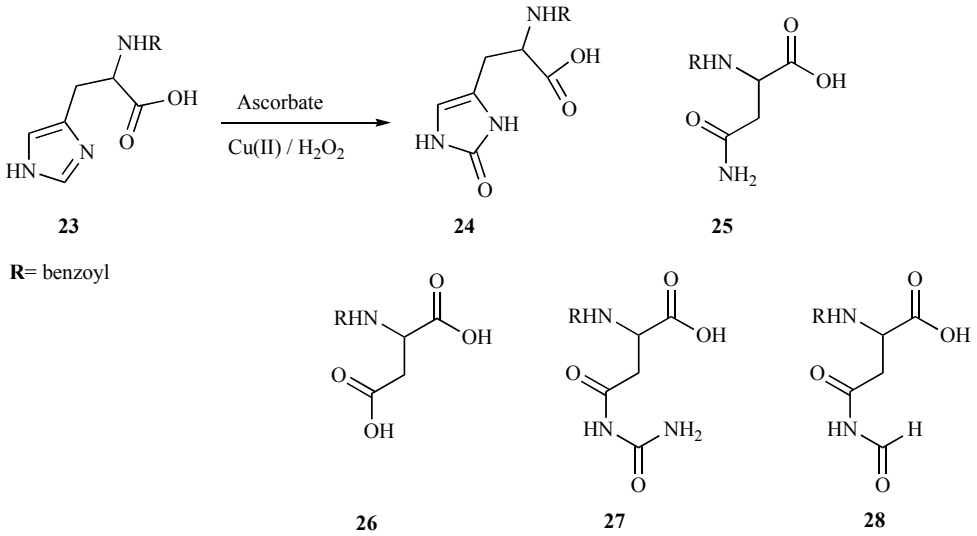
Several studies have been reported on the role of Cu(II) species in the site-specific oxidative modification of proteins and on the selective molecular recognition processes with amino acids residues able to increase the reactivity of the system [31]. For example, in the Alzheimer's disease, Cu(II) ions and O_2 could alter the redox state of amyloid α,β-peptides by oxidation of the methionine Met-35 residue to corresponding sulfoxide or sulfone analogues [32]. A similar effect was observed in other neurodegenerative diseases [33]. The amino acid residues, more susceptible to Cu(II) catalyzed oxidation, are His, Arg, Lys, Pro, Met and Cys [34]. The selectivity of these oxidations being modulated by the properties of the complexes is formed between the amino acid residues and the metal atom [35]. In the case of *N*-benzoyl-β-(2-oxo-imidazolonyl) alanine **23**, treatment with ascorbate and Cu(II) ions yielded **24** as the main reaction product, besides low amount of *N*-benzoyl asparagines **25**, *N*-benzoyl aspartic acid **26**, *N*-benzoylaspartic urea **27** and *N*-benzoyl-N^1-formyl asparagines **28** (Scheme **6**) [36].

Benzamide was also detected as a minor product suggesting the occurrence of C(α)-C bond oxidative scission processes. Note that the role of ascorbate in this transformation is to reduce Cu(II) to Cu(I), this latter ion is able to generate $HO^{\bullet}$ from H_2O_2 *via* a Fenton-like reaction [37]. Newly generated $HO^{\bullet}$ adds on the histidyl moiety with the formation of an heterocycle radical intermediate that can be further oxidized to **24** (Scheme **7**).

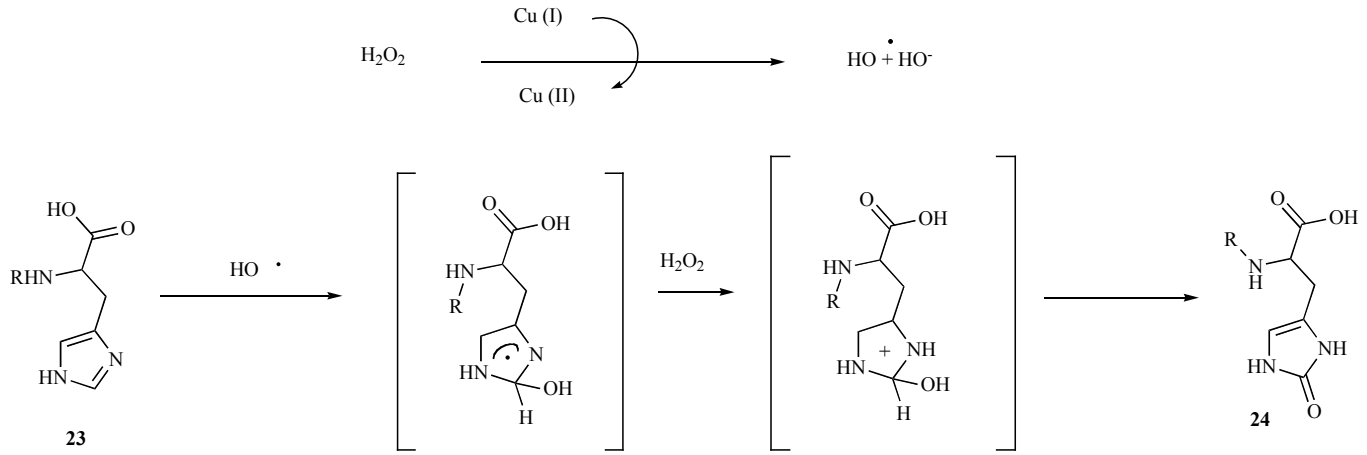
Compounds **25**-**28** are derived from **24** by a sequence of imidazole ring-opening and hydrolytic processes. In the case of the oxidation of the peptide backbone, the reaction might be initiated by hydrogen abstraction of the α-carbon with the formation of a carbon-centered radical "**A**". This intermediate further reacts with dioxygen to give a peroxy intermediate "**B**". Successive formation of a Schiff's base ("**C**") followed by elimination yields benzamide (Scheme **8**) [38].

The same procedure applied to peptides containing histidyl residues afforded site-specific cleavage, the N-terminal residue being more reactive than those in the second or third position [39]. The oxidation of proline (Pro) residue has also been studied. Treatment of **29** with the Cu(II)/H_2O_2 system provided three products, the 2-pyrrolidone **30**, deriving from an oxidative decarboxylation process, 4-hydroxy-2-pyrrolidone **31** and pyroglutamic acid **32** (Scheme **9**) [40].

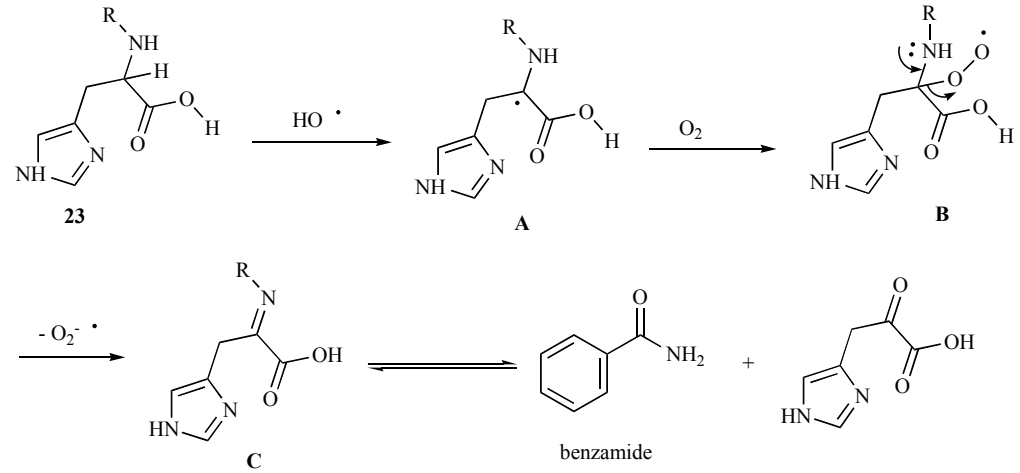
Hydroxyl radical-mediated hydrogen abstraction at α-carbon followed by an addition of dioxygen with the formation of peroxyl intermediate and successive scission of



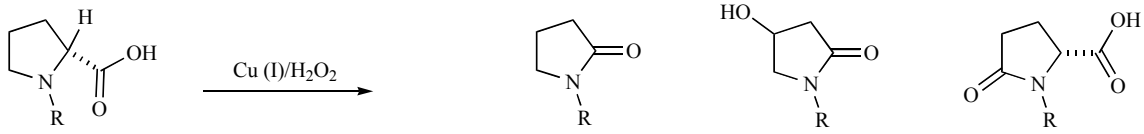
Scheme 6.



Scheme 7.



Scheme 8.



R= benzyloxy group

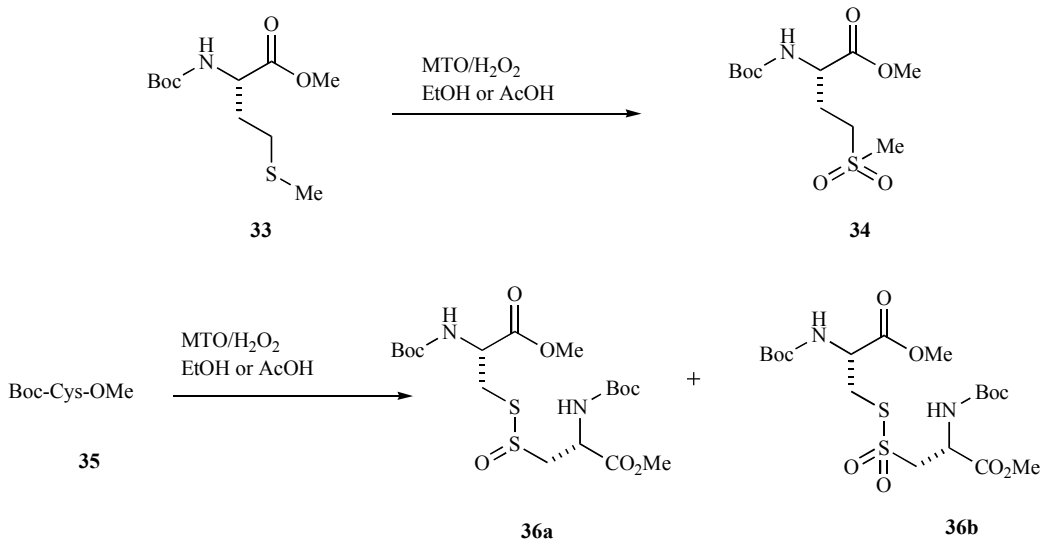
Scheme 9.

the C^{α}-C bond yields **30**. This procedure was further applied to various prolyl peptides to characterize the oxidative cleavage of collagen. In this latter case, the detection of γ-aminobutyric acid (GABA) was correlated to hydrolysis of 2-pyrrolidone derivatives [41].

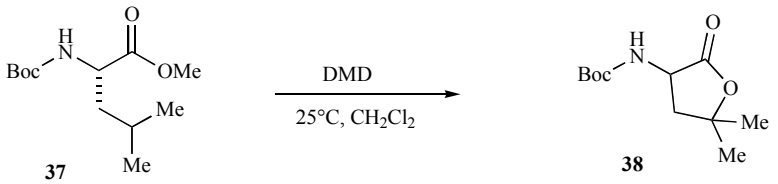
Procedures Based on Re^{VII}, Ozone, Dimethyldioxirane and 2-Iodoxybenzoic Acid

Re^{VII} species, such as methylrhenium trioxide (MTO, $MeReO_3$), have been used for the oxidative side-chain transformation of α-amino acids and peptides. For example, Boc-Met-OMe **33** treated with MTO/H_2O_2 system in EtOH or acetic acid at room temperature, gives the corresponding sulfone **34** in high yield (Scheme **10**). Under similar experimental conditions, the oxidation of cysteine derivative Boc-Cys-OMe **35** afforded the mono-sulfoxide **36a** or the sulfone **36b**, the selectivity of the reaction depending on the amount of H_2O_2 and on the nature of the solvent [42]. In these oxidations, the reactive intermediates are a monoperoxo $[MeRe(O)_2O_2]$ and a bis-peroxo $[MeReO(O_2)_2]\eta^2$-rhenium complexes produced by reaction of MTO with H_2O_2 [43]. Similar products were obtained during the ozonation of methionine in water [44]. Moreover, the interfacial ozonolysis of cysteine yielded cysteine sulfenate ($CysSO^-$), cysteine sulfinate ($CysSO_2^-$), and cysteine sulfonate [45].

The MTO/H_2O_2 system was highly selective in the oxidation of Met containing peptides, the Met residues being



Scheme 10.

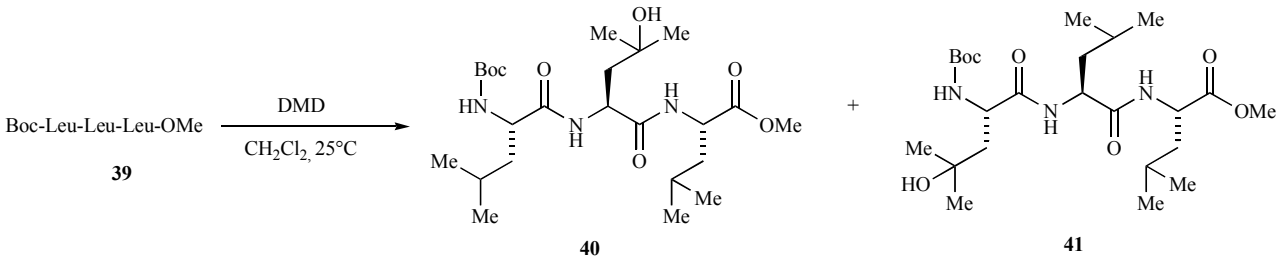


Scheme 11.

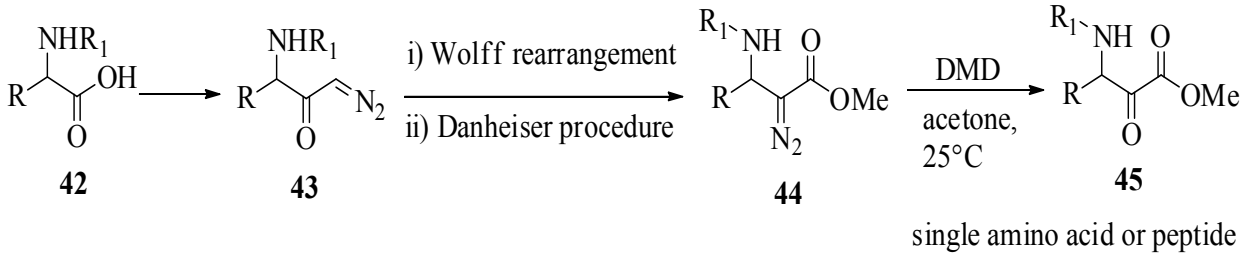
oxidized faster than other amino acids like Val, Leu, Ile, Pro, Ser, Tyr, Thr and His. In the series studied, triptophane (Trp) was the only reactive residue to afford the corresponding 3(2-oxo-2,3-dihydro-1H-indol-3-yl)alaninate derivative (not shown) [46]. Selective oxidation of sulfur containing amino acids is an important tool for the chemical engineering of proteins as a probe for binding domains [47] or to increase the biological activity [48]. Side-chain modification of high redox potential amino acid residues can be also performed by use of 3,3-dimethyldioxirane (DMD) [49]. The oxidation of protected leucine derivative Boc-Leu-OMe **37** with DMD in CH_2Cl_2 afforded the 4,4-dimethyl-4-butanolide derivative **38** as the only recovered product (Scheme **11**). Compound **38** was formed by oxygen atom insertion into the tertiary C-H σ bond in the Leu side-chain followed by spontaneous intramolecular cyclization [50]. Note that the reaction showed an high selectivity, the tertiary C-H σ-bond in the remote C(γ) position being the only oxidized site of the molecule. This selectivity is in accordance with a possible electronic deactivation of the more proximate α-CH σ-bond, due to the amino group [51].

Moreover, the reactivity of the Leu toward DMD in peptides was finely tuned on the basis of the position of the Leu residue in the sequence. In particular, *C*-terminal Leu residues were more reactive than *N*-terminal residues in dipeptides. This pattern was completely reversed in the oxidation of tripeptides, in which case the central Leu was functionalized (the modification of the *N*-terminal Leu was also found in very low amount). As a selected example in Scheme **12** is reported the oxidation of the tripeptide Boc-LeuLeuLeu-OMe **39** with DMD to yield side-chain modified peptides **40**-**41**. In this latter case the oxidation of the *C*-terminal Leu was not operative even in the presence of a large excess of the oxidant and for longer reaction time [52].

Oxidative modification of the amino acid and peptide backbone with DMD was also reported in the synthesis of biologically active β-amino-α-keto ester derivatives. These



Scheme 12.

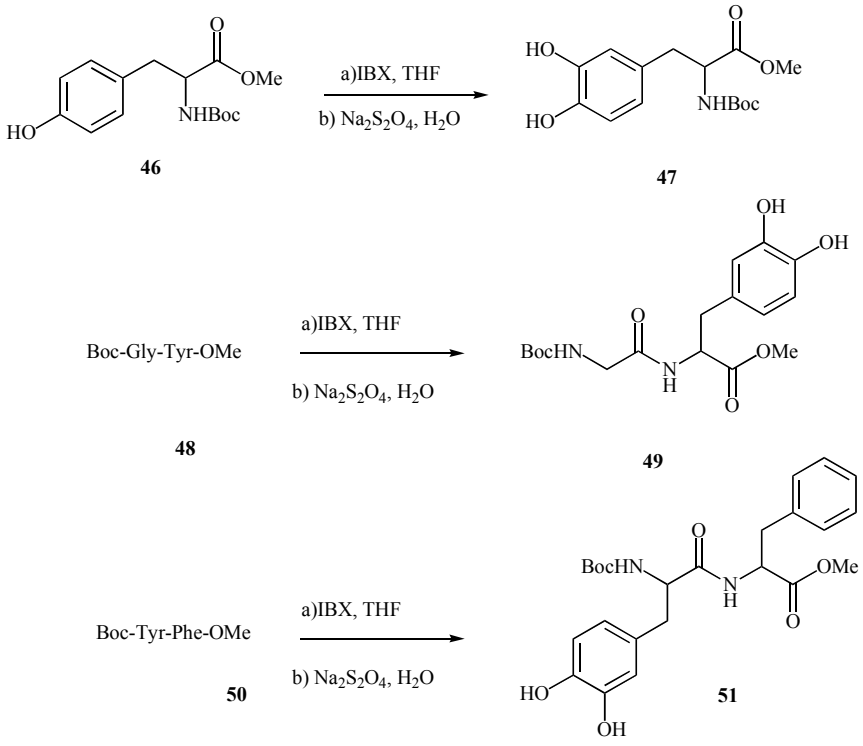


Scheme 13.

compounds are potent inhibitors of the serine proteinase chymotrypsin [53], and when embedded into peptides are potent and selective inhibitors of, respectively, cysteine proteinases calpain and cathepsin B [54], serine proteinases neutrophil elastase and cathepsin G [55], and the aspartyl proteinase pepsin [56]. An alternative synthetic approach to these ketoester, to circumvent the problem of racemization processes, requires the conversion of the *N*-protected amino acid (or peptide) **42** into the corresponding α-diazoketone **4**3, followed by Wolff rearrangement and Danheiser procedure to give the β-amino-α-diazo ester derivative **44**. To complete the synthesis, the diazo group of **44** was oxidized with DMD under neutral conditions to afford desired α -keto-β-amino methyl ester derivative **45** (Scheme **13**) [57].

Some of new peptide derivatives showed a potent inhibitory activity against bovine α -chymotrypsin and porcine pancreatic elastase [58].

Recently, Harding and co-workers reported the oxidation of threonine (Thr) residues with 1-hydroxy-1-oxo-1H-1λ5-benzo[d][1,2]iodoxol-3-one (2-iodoxybenzoic acid, IBX) as a synthetic strategy to incorporate an aldehyde or ketone moiety into a peptide chain [59]. Among some other possible applications of IBX, the *ortho*-hydroxylation of phenols to catechols with a regioselectivity similar to that of natural polyphenol oxidases, has been reported [60]. As an extension of this procedure, an efficient route to 3,4-dihydroxylphenylalanine (DOPA) **47** and DOPA peptides was described by oxidation of *L*-tyrosine **46** and *L*-tyrosine derivatives, with IBX (Scheme **14**). DOPA was obtained after reduction of corresponding *ortho*-quinone with sodium dithionite. Oxidation reactions proceeded in good yields and high chemo- and regio-selectivity irrespective to the position of Tyr residue in the sequence of the peptide. As a selected example, the oxidation of Boc-Gly-Tyr-OMe **48** with IBX afforded Boc-Gly-DOPA-OMe **49** in high yield. In a similar way, treatment of Boc-Tyr-Phe-OMe **50** gave the corresponding Boc-DOPA-Phe-OMe **51** in satisfactory conversion and yield (Scheme **14**) [61]. The chirality of the DOPA residue was retained under the reaction conditions. The efficiency and the selectivity of the reaction were successfully tested also using recyclable polymer supported IBX.

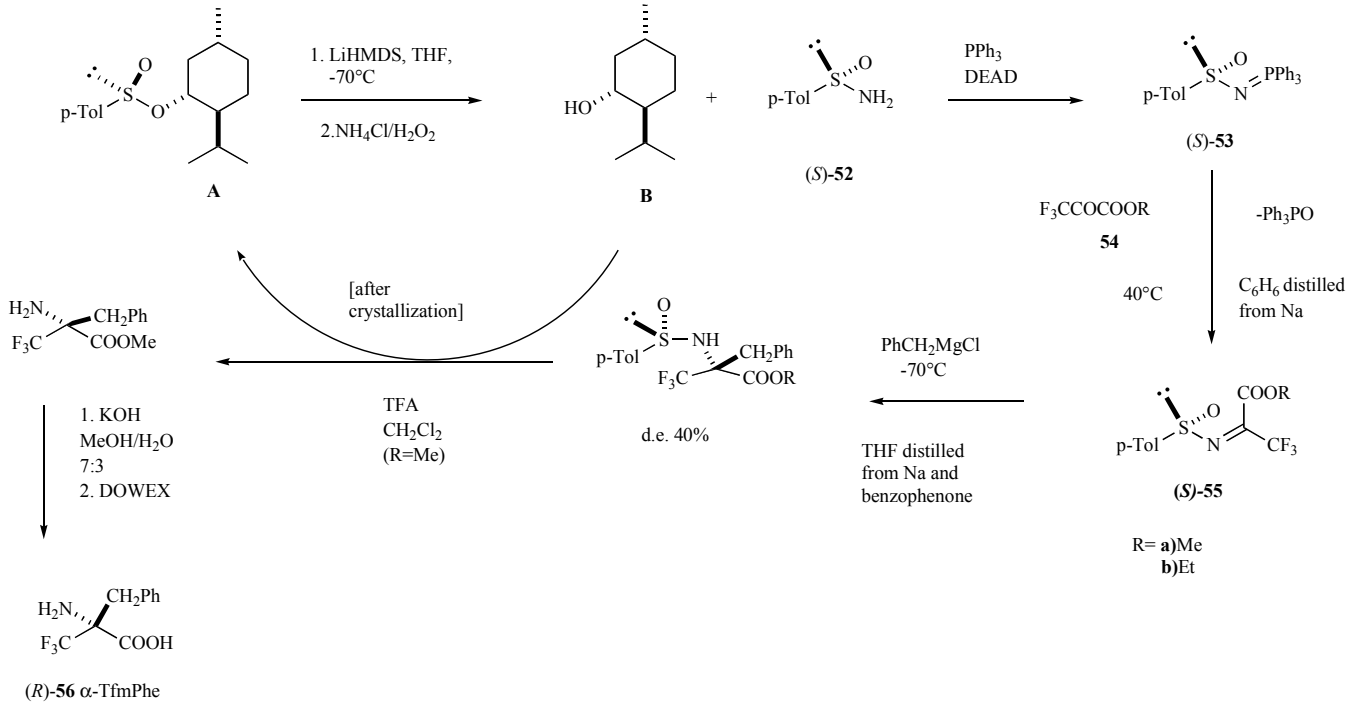


Scheme 14.

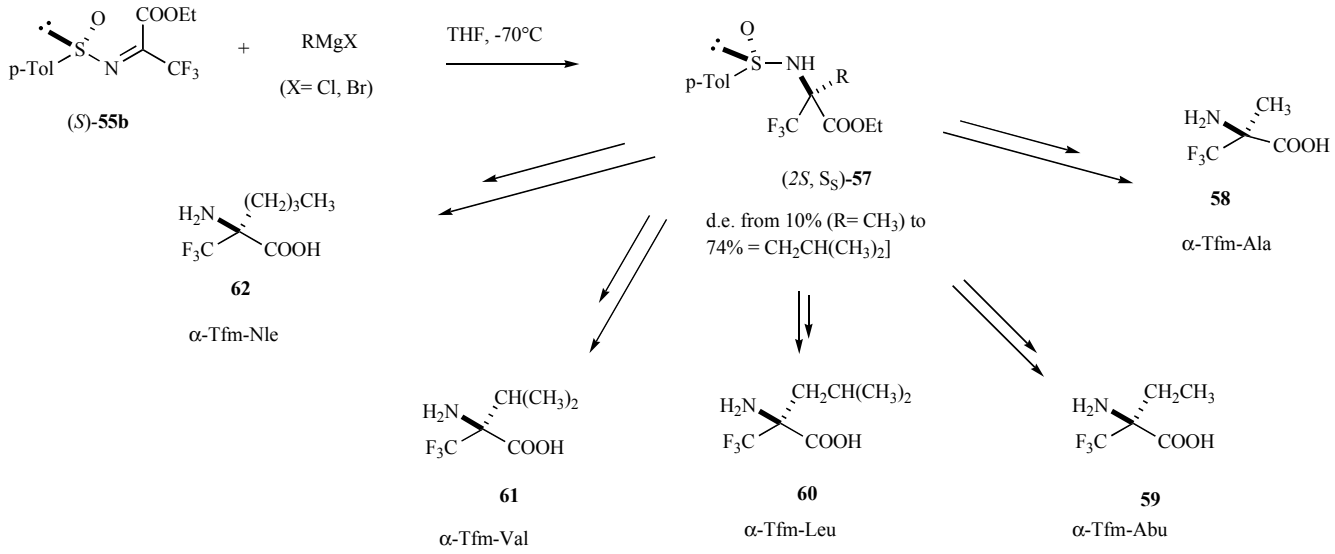
Fluorinated Analogues of Amino Acids and Peptides by Selective Side-Chain/Backbone Modifications

Organofluorine chemistry has undoubtedly known an astonishing growth, as witnessed by the large number of thematic articles, reviews and books appeared in literature, in the last two decades [62]. Due to its small steric size, high electronegativity and carbon-fluorine bond strength, fluorine is able to bring about striking, and often unexpected, changes in physico-chemical properties, reactivity and biological features of organic molecules. Also nature, while being able to synthesize a large number of halogen-containing natural products failed to handle fluorine, producing only a dozen fluorinated molecules most of whom are very toxic for living organisms (the toxicity of monofluoroacetic acid, is well known).

A limited number of high plants and bacteria are the only living organisms able to metabolise inorganic fluoride: so, fluoroorganic compounds can be full regarded as practically xenobiotic substances [63]. An exception to this rule stems from the pioneering work of O'Hagan *et al*., who discovered the first fluorinating enzyme from the microorganism *Streptomyces cattleya*, and resolved its X-ray structure, providing an exciting insight into the mechanism of biocatalyzed organofluorination with inorganic fluoride [64]. In bioorganic and medicinal chemistry, the selective introduction of fluorine atoms or suitable fluorinated functions into a molecule has become a method of choice in order to modify and tune its biological properties [65]. For example, a fluorine atom has been used with great success as a replacement for either, a hydrogen atom or a hydroxy group, while a CF_2 has been used as a mimic for an oxygen atom. By the same way, xenobiotic trifluoromethyl (Tfm, CF_3) group is well recognized as a substituent of distinctive qualities: indeed, it is simultaneously highly hydrophobic, electron-rich and sterically demanding; in addition, it can provide high stability *in vivo* and shows a good mimic with several naturally occurring residues such as methyl, isopropyl, phenyl, and others [66]. In addition, the sensitivity of ^{19}F NMR spectroscopy along with large ^{19}F–^{1}H coupling constants, renders fluorine incorporation of a particularly powerful tool for the investigation of biological processes [67]. The combination of the unique physical and chemical properties of fluorine with proteinogenic amino acids, represents a new approach to the design of selected analogues of naturally occurring bioactive compounds, including peptides, with improved pharmacological properties. In particular, it has been demonstrated, most notably by the groups of Kumar [68], Koksch [69] Ulrich [70] and Seebach [71], that selective incorporation of fluorinated amino acids allows for remarkable opportunities to study and control the dynamics of peptide secondary structure and folding. Moreover, examples describing the direct regiospecific fluorination of selected amino acid containing peptides to evaluate the effect on biological activity [72], as well as parallel incorporation of different fluorinated amino acids with the aim to obtain new "Teflon" proteins [73], have been also published. An improvement of the therapeutic profile can be achieved by replacement of selected proteinogenic amino acids, in strategic positions, with unnatural building blocks by incorporation of fluorinated amino acids. So, protein (peptide) design and engineering of fluorinated amino acids have achieved remarkable progress and the systematic investigation of the interaction properties of fluoroalkyl groups in a native polypeptide environment, broadens the scope of fluorinated amino acids to the rational design of structural motifs and protein interfaces. It follows that further progress in this area



Scheme 15.



Scheme 16.

of research might depend on availability of various structural and functional types of fluorinated amino acids. Over the past twenty years, substantial progress has been made in the development of general approaches for the preparation of fluorine containing amino acids, also in the enantiomerically pure form [74].

New Synthetic Strategies. Fluorinated α,α-Disubstituted-α-Amino Acids

We have grouped the fluorinated amino acids into three main types: fluorinated α,α-disubstituted-α-amino acids, fluorinated α-amino acids and fluorinated β-amino acids. In addition, a brief discussion on the synthetic routes available for the preparation of fluorinated amino acids for radiopharmaceutical applications, is presented.

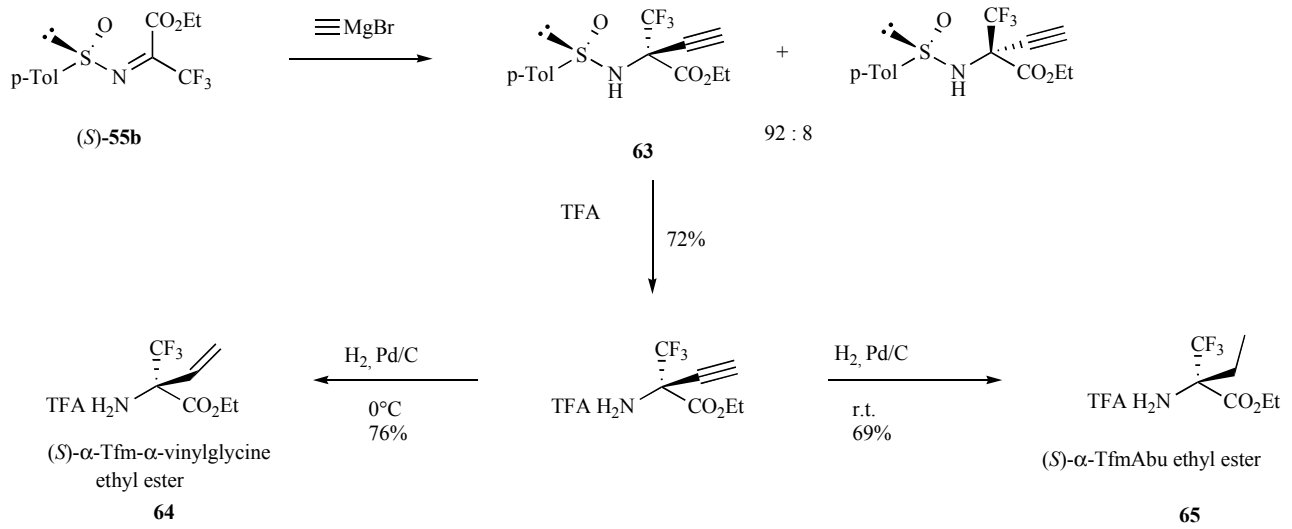
α-Trifluoromethyl (α-Tfm) and α-difluoromethyl (α-Dfm) amino acids represent a special class of α-amino acids. Generally, the α,α- dialkylation of amino acids leads to the stabilization of certain secondary structure motifs, while the fluorination process induces an alteration of the whole molecule. In fact, fluorine increases the electronegativity, lipophilicity, and the steric demand of the molecule (these properties non-linearly increase with the number of fluorine atoms). Incorporation of α-fluoroalkyl amino acids into peptides can retard proteolytic degradation and enhance *in vivo* absorption as well as drug permeability. Stabilization of secondary structure motifs and enhancement of thermal stability of peptides were also observed. Nevertheless, it must be pointed out that, the fluorine effect strictly depends on the position of the fluoroalkyl-substituent in the peptide. An interesting and efficient approach to obtain α-Tfm α-amino acids, exploiting an enantioselective process, has been based on the use of highly electrophilic sulfinimines [75]. These compounds were prepared by the aza-Wittig reaction of the chiral Staudinger reagent **53** (synthesized from the Davis sulfinamide **52**) with ethyl or methyl trifluoropyruvates **54** (Scheme **15**).

The obtained sulfinimines (*S*)-**55** are much more stable towards hydrolysis compared to corresponding *N*-acyl and *N*-alkoxycarbonyl derivatives. Moreover, the chiral auxiliary can be easily recovered as menthyl sulfinate **A** allowing the cost-effective preparation of non-racemic α-Tfm amino acids on a multi-gram scale. The sulfinimines (*S*)-**55** were reacted with a wide range of Grignard reagents to produce a library of α-Tfm amino acid derivatives (Scheme **15**: α-Tfm Phe, **56**; Scheme **16**: α-Tfm Ala, **58**; α-Tfm Abu, **59**; α-Tfm Leu, **60**; α-Tfm Val, **61**; α-Tfm NLe, **62**). The diastereoselective outcomes depend on the nature of the Grignard reagent. Normally, sterically hindered nucleophiles gave the best results providing diastereomeric excesses (d.e.'s) up to 74%.

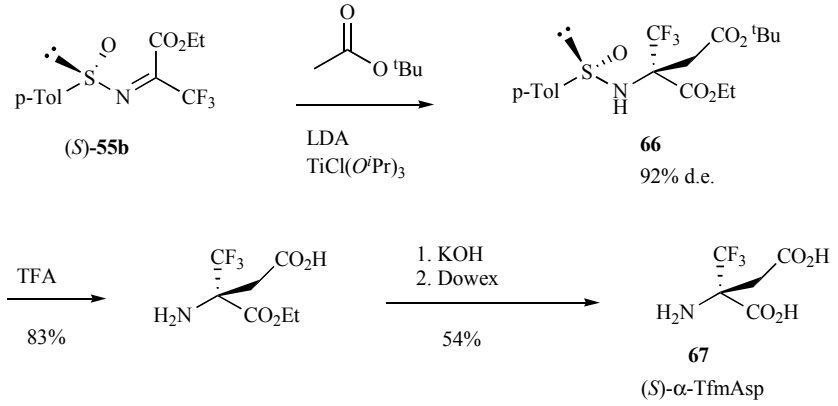
It is worth noting that, the reaction of (*S*)-**55** with vinyl and phenylmagnesium halides resulted in the complete addition of the Grignard reagent to sulfur atom. Nevertheless, α-Tfm-α-vinylglycine ethyl ester **64** could be synthesized by addition of ethynylmagnesium bromide to sulfinimine (*S*)-**55b** and subsequent chemoselective reduction of the triple bond [76] (Scheme **17**). The addition of the ethynyl Grignard to (*S*)-**55b** occurred with surprisingly high diastereocontrol (84% d.e.). Afterwards, cleavage of the chiral auxiliary and hydrogenation of the major diastereomer **63** provided access to ethyl esters of (*S*)-αTfm-α-vinylglycine **64** (76%) and (*S*)-αTfm-α-aminobutyric acid (αTfmAbu) **65** (69%).

Chiral sulfinimine (*S*)-**55b** was also used as a template to prepare α-TfmAsp **67** *via* a diastereoselective Mannich-type addition [77]. Different reagents were screened for the generation of the metal enolate of *tert*-butyl acetate to carry out an additional step. The use of both LDA for the lithiation and $TiCl(O\text{-}iPr)_3$ to obtain the subsequent transmetallation, gave the best results, and furnished diastereomer **66** in 92% d.e. The d.e. could even be raised to 97% by crystallization. Finally, (S)-α-TfmAsp **67** was released by a deprotection sequence (Scheme **18**).

Interestingly, an additional step to chiral sulfinimine (*S*)-**55b** was also performed with further methylene active



Scheme 17.



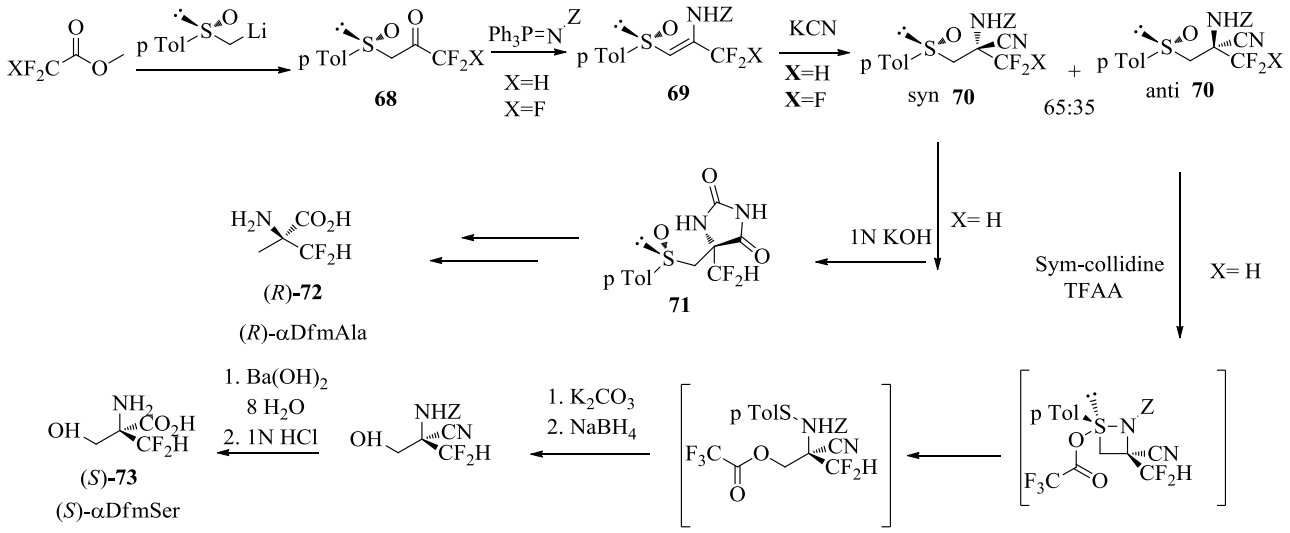
Scheme 18.

nucleophiles, even though a decrease of selectivity was observed [78]. It is well known that chiral sulfoxides are important building blocks in organic synthesis; indeed, they have been used as suitable auxiliaries for the preparation of optically pure α-Dfm α-amino acids. α-(fluoroalkyl)-β-sulfinylenamines **69**, readily prepared from α-fluorinated-α'-sulfinyl ketones **68** *via* Staudinger (aza-Wittig) reaction with triphenyliminophosphoranes, were used as valuable templates for the asymmetric Strecker reaction (Scheme **19**) [79]. The α-fluoroalkyl substituent and the β-sulfinyl group render the carbon C-2 highly reactive towards nucleophiles. The hydrocyanation of β-sulfinylenamines was achieved in high yields, affording the *syn*-product **70** as the major diastereoisomer (Scheme **19**).

Even if the diastereoselectivity of hydrocyanation was not satisfactory, pure amino nitriles **70** were easily obtained after chromatographic separation. The removal of the chiral auxiliary sulfinyl group by reductive desulfurization of sulfinylmethylene hydantoin **71**, allowed the preparation of amino acid α-Dfm Ala (*R*)-**72**. Alternatively, sulfinyl group could be replaced by an oxygen atom, under non-oxidative Pummerer rearrangement conditions to give α-Dfm Ser (*S*)-**73**. Noteworthy, α-Tfm and α-Dfm amino acids have been prepared in enantiomerically pure form. An exhaustive review describing the most frequently employed strategies for the synthesis of α-difluoromethyl and α-trifluoromethyl substituted α-amino acids, has been recently published [80]. As a particular case, conformationally constrained cyclic amino acids have recently gained considerable interest because of their ability to control the conformation of peptides for structure–activity relationships investigations as well as for the design of peptidomimetics. In particular, the incorporation of a proline unit is known to restrict the amino acyl-proline cis/trans isomerization, to limit the protein folding and consequently to modulate the biological activity of peptides. Selectively fluorinated proline-type amino acids and pyroglutamic acids derivatives, were also reported to be efficient tools for the control, as an example, of the peptidyl bond geometry [81].

Side Chain Fluorinated α-Amino Acids

Frequently, in the planning of stereoselective processes, synthetic routes based on the use of an enantiopure auxiliary have been exploited successfully. An interesting example, was based on the diastereoselective alkylation, at low temperature, of Schiff bases **74a** and **74b** derived from (*R*,*R*,*R*)-2-hydroxy-3-pinanone and glycine *tert*-butyl ester or



Scheme 19.

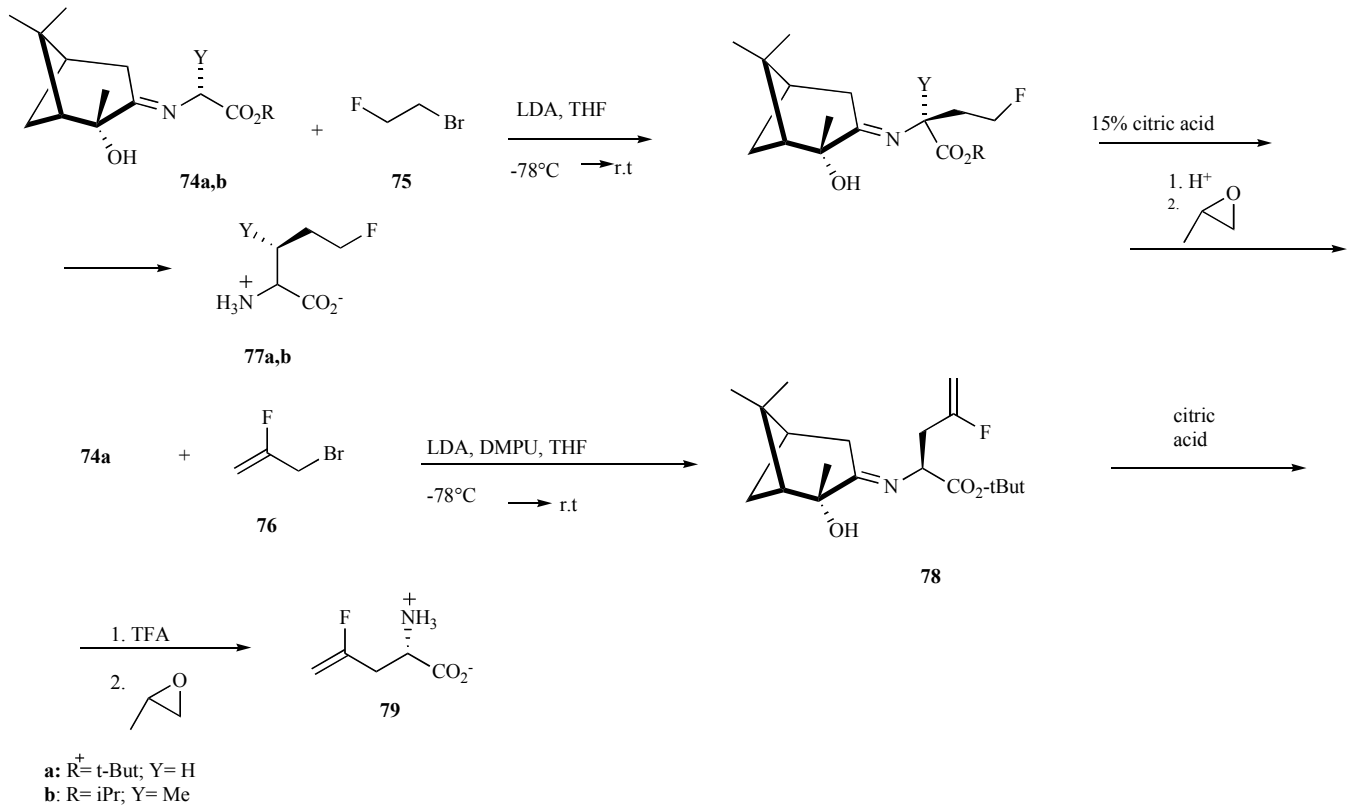
alanine isopropyl ester, respectively, with 1-bromo-2-fluoroethane **75** or 3-bromo-2-fluoropropene **76** (Scheme **20**) [82]. This stereoselective synthesis allowed the preparation of, respectively, (*S*)-2-amino-4-fluorobutanoic acid **77a** [>96% enantiomeric excess (e.e.)], α-methyl derivative (*S*)-**77b** (85% e.e.), and (*S*)-2-amino-4-fluoro-4-pentenoic acid **79** (81% e.e.). 3-Bromo-2-fluoropropene **76** was a very reactive and selective alkylation reagent (>95% e.e.). However, during hydrolysis of the alkylated product **78** a partial racemization occurred and the amino acid **79** was obtained with 81% of enantiomeric purity.

The first synthesis of enantiomerically pure γ-fluorinated glutamine was based on the side-chain fluorination of chiral (*R*)-Garner's aldehyde **80** in a Reformatsky reaction with ethyl bromodifluoroacetate, under ultrasonic conditions (Scheme **21**) [83]. The diastereomeric mixture of fluorinated alcohols **81** was converted into imidazolylthiocarbonates **82** which, in turn, were derivatized by deoxygenation and successive oxazolidine ring cleavage and oxidation, affording 4,4-difluoroglutamic acid derivative **83**. Aminolysis of ester **83** and deprotection of amino group afforded the target 4,4-difluoroglutamine **84**, in 80% yield and high e.e. (> 99%).

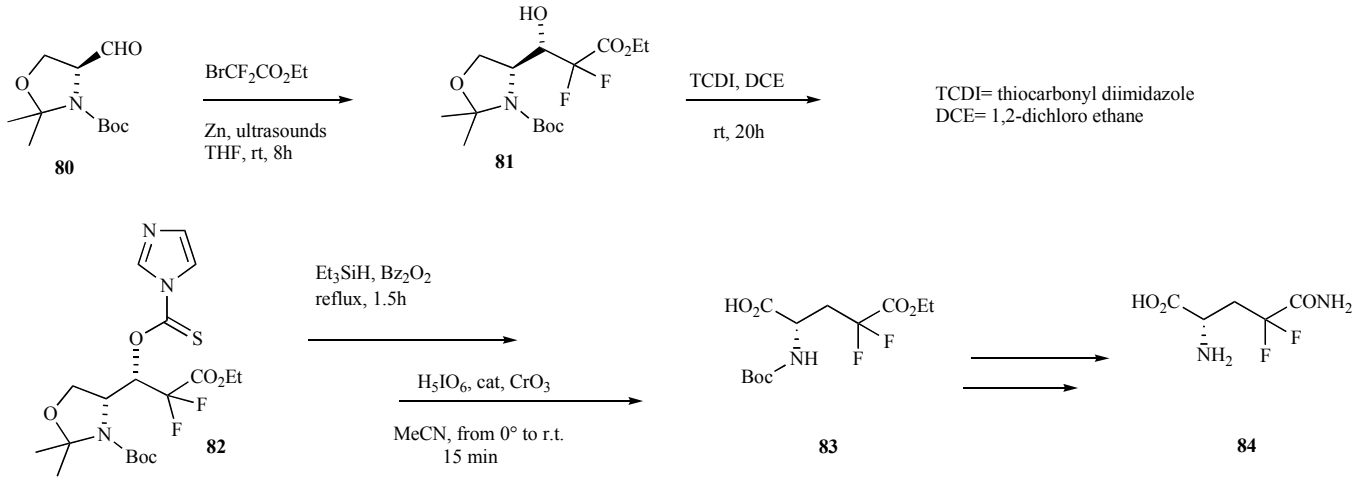
An efficient asymmetric method for the synthesis of γ-perfluorinated α -amino acids, based on photoinduced diastereoselective iodoperfluoroalkylation of acrylic acid derivatives, has been published few years ago [84]. Among different derivatives bearing chiral auxiliaries, the best results were obtained in the presence of *N*-acyloylcamphorsultam **85**. The addition proceeded smoothly in the presence of $Na_2S_2O_3$, under UV irradiation, affording an enriched mixture of diastereomers **86a** and **86b**, in good yields (Scheme **22**). The next displacement of iodide, by sodium azide, proceeded with inversion of configuration, and the azides **87a** and **87b** were obtained without any loss of stereochemical purity. After removal of the auxiliary, the expected γ -perfluorinated α -amino acids (*S*)-4,4,5,5,6,6,6-heptafluoronorleucine **88a** and (*S*)-4,4,5,5,5-pentafluoronorvaline **88b**, were obtained in satisfactory yields.

Recently, a very similar method for the access to γ-fluorinated α-amino acids, as racemic mixture, through the indium-mediated reductive radical addition of perfluoroalkyl iodides to dehydroamino esters, has been published by same authors [85]. Generally, two principal routes have been followed in order to obtain enantiomerically pure fluorinated compounds, namely the reaction of fluorinating agents on late precursors, or the use of relatively simple fluorinated substrates already possessing some stereogenic center, which, after appropriate elaborations, can afford the target product. Within these different approaches, examples are given for the preparation of chiral non racemic 3-fluoroalanine. In the first case, a convergent synthetic methodology has been developed to access both (*S*)- and (*R*)-3-fluoroalanine enantiomers and their corresponding *N*-methyl analogues, in optically pure form, through a common oxazolidinone intermediate **89** obtained from *L*- or *D*-serine [86]. The key fluorodehydroxylation step (*that is*, transformation of C-O to C-F moiety) of chiral intermediate was performed with HF-pyridine and Deoxo-Fluor [Bis(2-methoxyethyl)aminosulfur Trifluoride] [65b] (Scheme **23**, route a). In the second type of strategy, the (*S*) enantiomer of 3-fluoroalanine has been stereoselectively synthesized exploiting the "chiral sulfoxide chemistry". The key steps are being the azidation of the α-fluoro α'-sulfinyl alcohol **90**, under Mitsunobu conditions and the one-pot transformation of the *N*-Cbz α-sulfinyl amine **91** into *N*-Cbz aminoalcohol **92**, through a "non-oxidative Pummerer reaction" (Scheme **23**, route b) [87].

The two following syntheses demonstrate the utility of a commercially available and optically pure chiral substrate, the (*R*)-2,3-*O*-isopropylideneglyceraldehyde **93**, for the synthesis of a wide variety of fluorinated amino acids. In both cases, the key fluorinating step has been performed by nucleophilic fluorinating reagents. In the first example, fluorinated analogues of Ser and Thr were obtained starting from glyceraldehyde acetonide **93** or from its derivative **95**, respectively, being diethylamino sulfurtrifluoride (DAST) the same fluorinating agent used. The final (2*R*)-*β*-



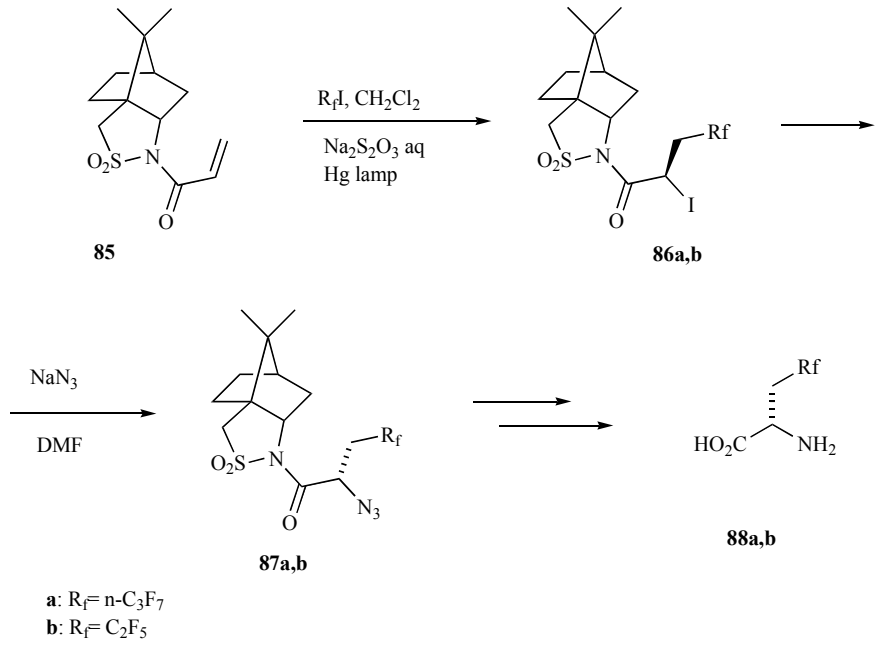
Scheme 20.



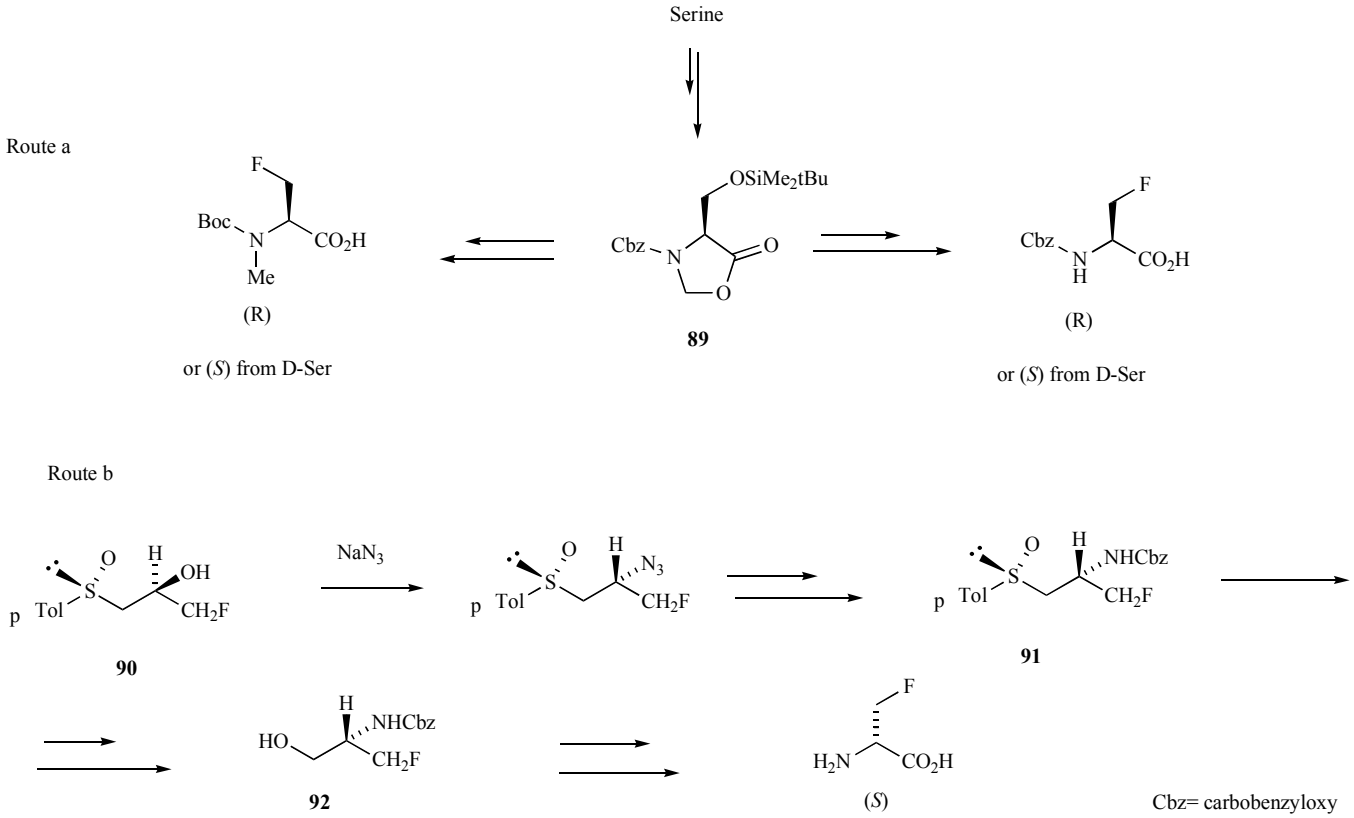
Scheme 21.

difluoroalanine **94** and (2*S*,3*S*)-*γ*-difluorothreonine **96**, appropriately protected for use in Fmoc-based solid-phase peptide synthesis, were obtained after usual synthetic modifications (Scheme **24**, route a) [88]. In the second example, the stereocontrolled synthesis of *N*-protected (2*S*,3*R*)-2-amino-3-fluoroundecanoic, and (3*R*)-3-amino-2,2-difluoroundecanoic acids, **97** and **98**, respectively, was performed starting from glyceraldehyde acetonide **93** by Mitsunobu reaction for the introduction of amino function and incorporation of fluorine atom(s) by morpholinotrifluorosulfurane (Morpho-DAST) as fluorinating agent (Scheme **24**, route b) [89].

Recently, the stereoselective syntheses of fluorinated analogues of proteinogenic amino acids like Val and Leu, have been published [90]. Starting from commercially available (*E*)-4,4,4-trifluoro-3-methylbut-2-enoic acid **99**, the (2*S*,3*S*)-4,4,4-trifluorovaline **105** and (2*S*,4*S*)-5,5,5-trifluoroleucine **109** have been prepared, by *N*-acylation of Oppolzer's sultam **100** (in order to control the CF_3-substitution during the successive reduction to sultam **101**), conversion of the major diastereomer (3*S*)-**101** to **103**, SeO_2-promoted oxidative rearrangement (to afford the dihydro-2H-oxazinone **104)**, and face-selective hydrogenation of the C=N double bond, followed by hydrogenolysis-hydrolysis

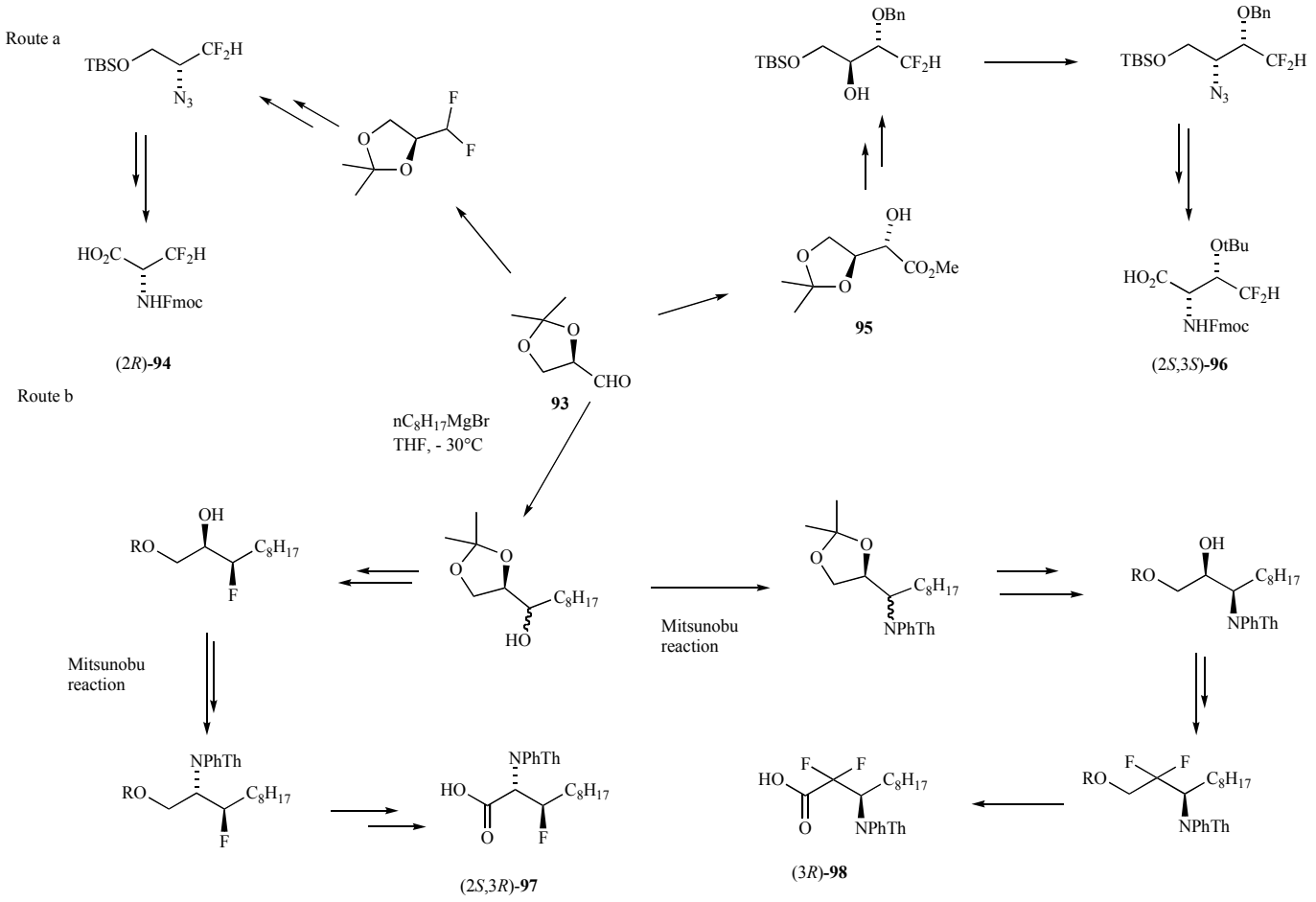


Scheme 22.



Scheme 23.

(Scheme **25**, route a). Sultam 3*S*-**101** served as the common starting material for preparation of both fluorinated amino acids **105** and **109**; indeed, the synthesis of (2*S*,4*S*)-5,5,5-trifluoroleucine **109** followed a similar sequence as **105**, provided that the homologous carboxylic acid **106** be obtained before to follow the same synthetic sequence already described (Scheme **25**, route b). The latter method may be applicable to the synthesis of any of the four diastereomers of **105** and **109**, by appropriate choice of the chiral auxiliaries' configurations: the Oppolzer sultam **100** (for the CF_3 stereocentre) and the phenylglycinol **102** (for the C2 stereocenter). The main drawback of this sequence is the



Scheme 24.

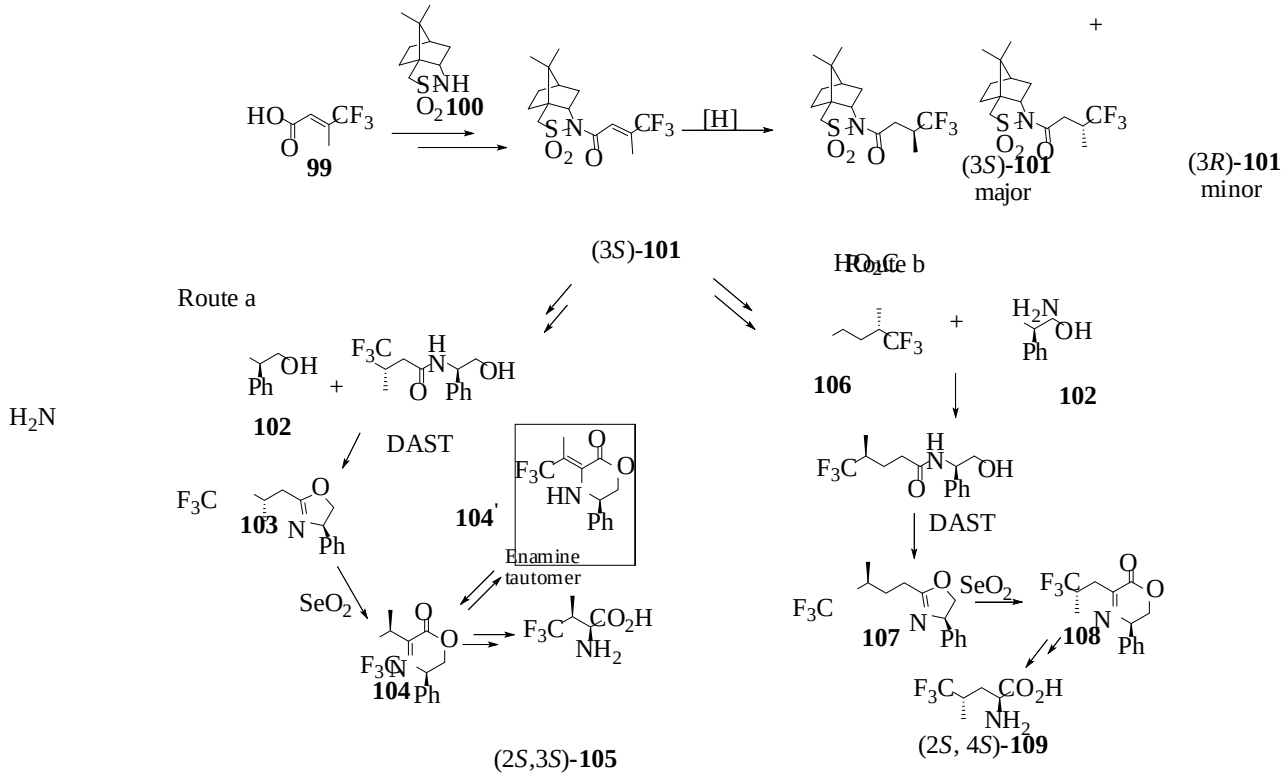
partial epimerization at the α-stereocenter of oxazinone **104**, likely due to the presence of traces of acidity (formed during the oxidative transformation of **103** to **104**), that may catalyze the imine-enamine isomerization (from **104** to **104'**).

Among different types of fluorinated α-amino acids, linear ω-trifluoromethyl-containing α-amino acids are of considerable interest due to the peculiar properties of trifluoromethyl group, like strong steric and electrostatic requirements. The asymmetric synthesis of linear ω-trifluoromethyl-containing amino acids *via* alkylation of chiral equivalents of nucleophilic glycine and alanine, has been published [91]. The key-step is based on the asymmetric alkylation reactions between the nickel(II) complex of the Schiff base of glycine (or alanine) with (*S*)-*ortho*-[*N*-(*N*-benzylprolyl)amino]benzophenone **110a** (or **110b**) and ω-trifluoromethyl alkyl iodides **111** (d. r. ranging from 94 to 99%) to afford linear ω-trifluoromethyl-containing α-amino acids **112a** (R = H) and previously unknown α-methyl derivatives **112b** (R = Me). The chiral ligand (*S*)-**BPB** used in the transformation was quantitatively recovered (Scheme **26**).

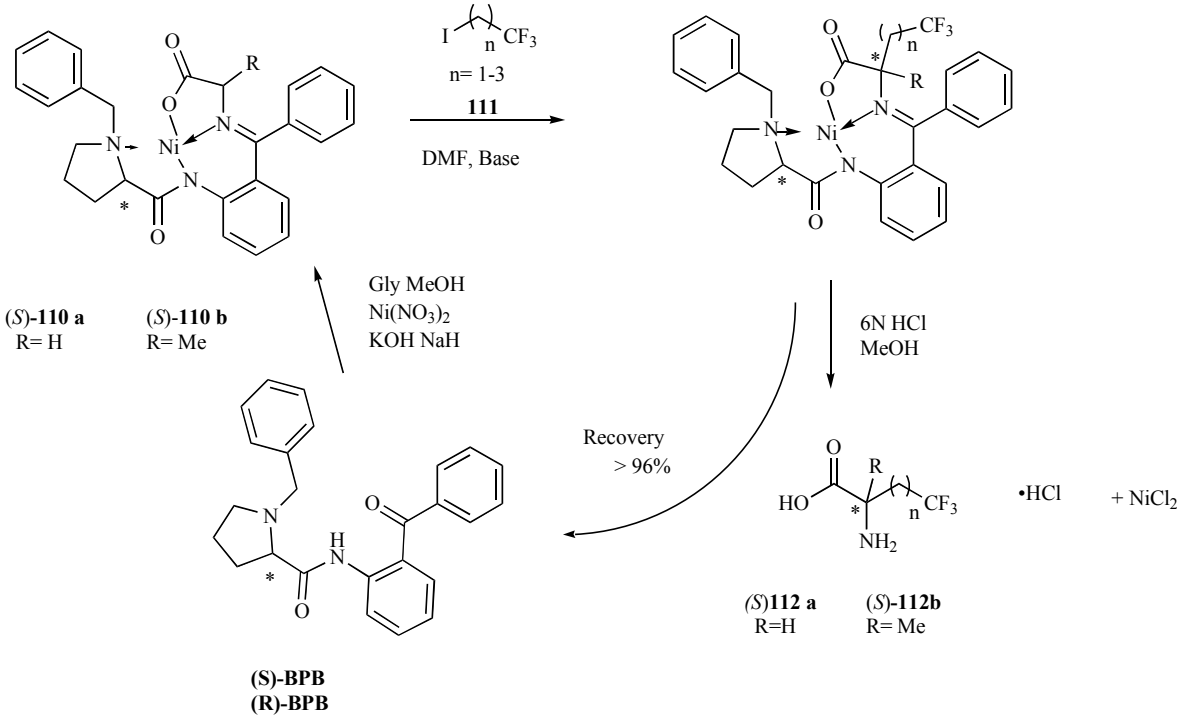
Side Chain Fluorinated β-Amino Acids

β-Amino acids are important components of bioactive natural products and peptidomimetics and they can enhance resistance to proteolysis. Peptides derived from β-amino acids possess fascinating and well-defined secondary structure; in some cases, their backbone structures are able to mimic α-peptidic hairpin turns [92]. In addition, some of these β-amino acids exhibit antimicrobial activity, and they are useful synthetic precursors to lactam antibiotics and other unnatural oligopeptides. Two main strategies have been, so far, investigated, namely selective fluorination of appropriate functional groups in β-amino acid frameworks, and exploitation of already fluorinated small molecules for their use as synthetic building blocks [93]. Recently, a methodology for the enantioselective synthesis of α-fluorinated β^2- and β^3-amino acids (Fig. **1**) has been developed in order to investigate and extend the effectiveness of fluorinated β-amino acids to act as transition-state-analogue based inhibitors of serine proteases [94].

The preparation of α-fluoro-β^2-amino acids was realized starting from readily available carboxylic acids **113** (Scheme **27**, route a). The successive conversion to the Evan's oxazolidinone **114** followed by two diastereoselective steps, namely, fluorination with *N*-fluorobenzenesulfonimide (NFBS) [95], and alkylation with benzyl chloromethyl ether, gave substrate **115** in high diastereomeric excess (>95%). Subsequent removal of the oxazolidinone and amination at the Bn-protected hydroxyl center gave optically active



Scheme 25.



Scheme 26.

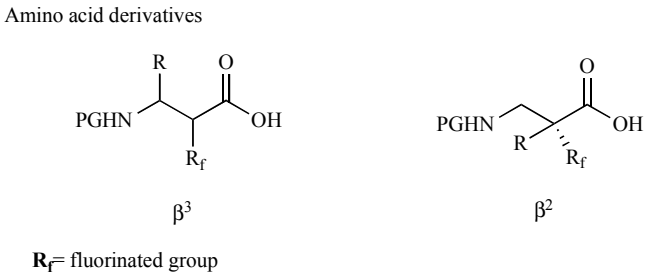
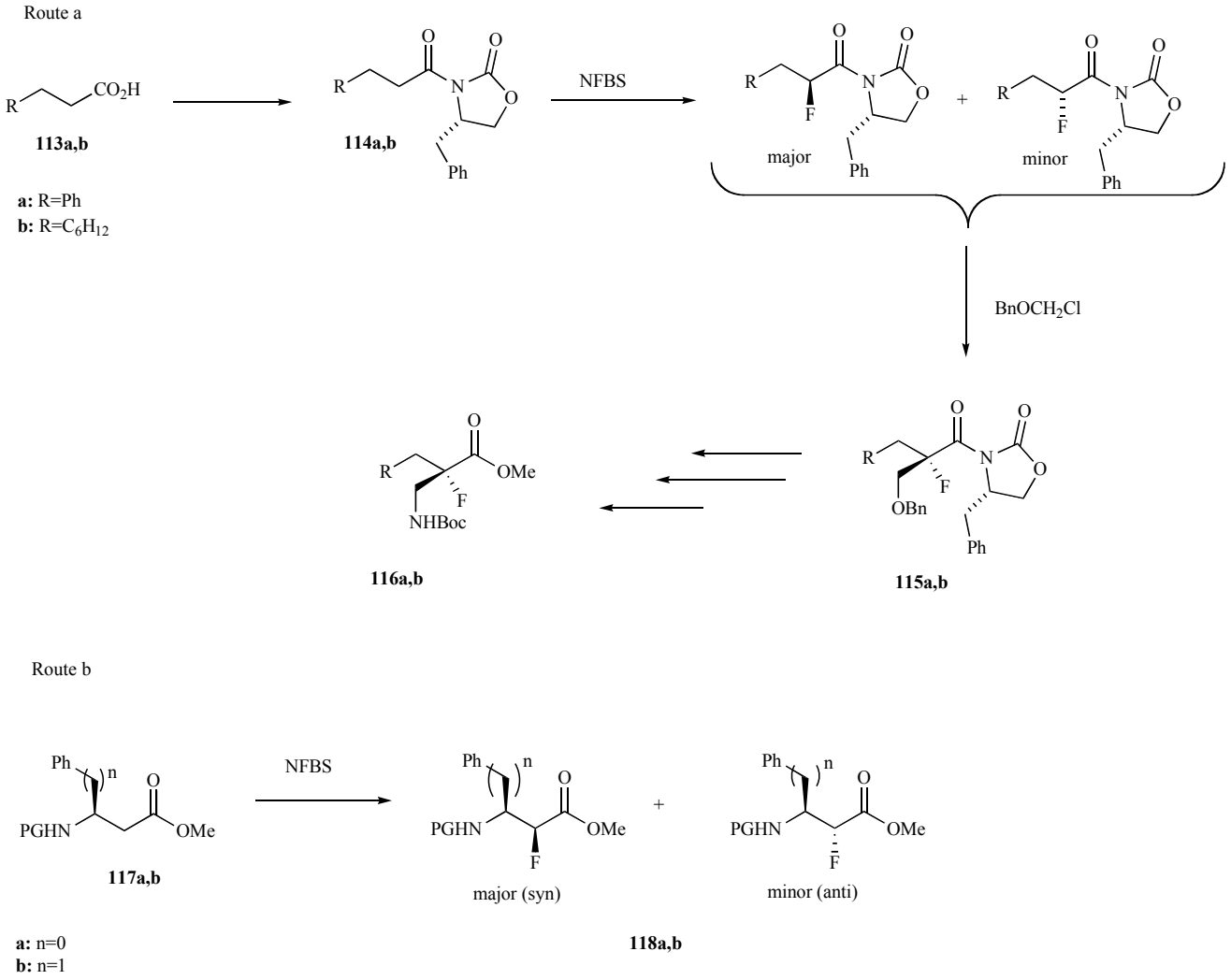


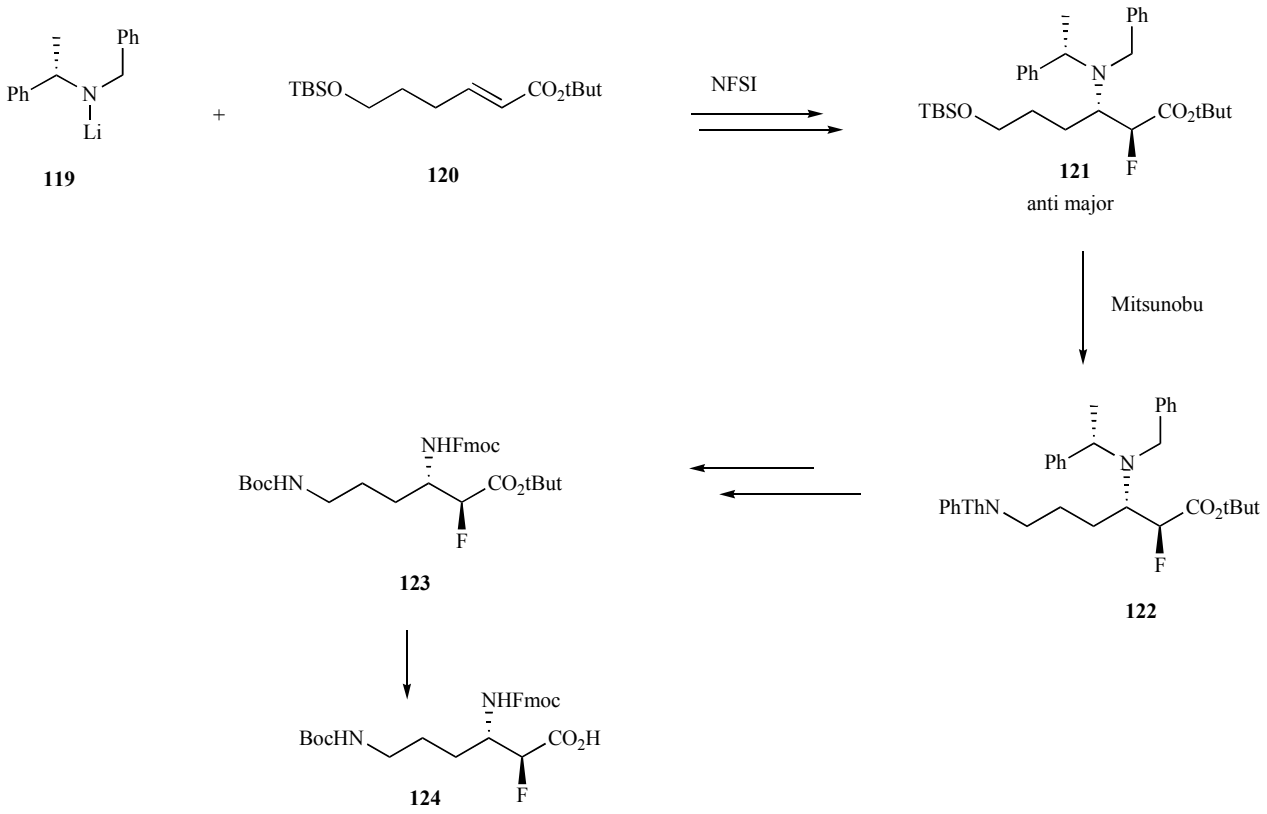
Fig. (1).



Scheme 27.

α-fluorinated β^2-amino acid methyl esters **116a** and **116b** (Scheme **27**, route a).

Differently, α-fluoro-β^3-amino acids **118a** and **118b** derivatives were obtained by a simple stereoselective fluorination, with *N*-fluorobenzenesulfonimide (NFBS) of the corresponding β^3-amino acids **117a,b**, which are readily obtained by Arndt-Eistert homologation of an optically active α-amino acid. The diastereoselectivity of fluorinating step [d.e. ranging from 66% (for **118a**) to 90% (for **118b**)] ranges from only moderate to good values, depending from both the length of the alkyl chain on the β-carbon, and the nature of *N*-protecting group (Scheme **27**, route b). An interesting procedure is based on a tandem conjugate addition-fluorination sequence performed on α,β-unsaturated esters with enantiopure lithium amide derived from (*S*)-*N*-benzyl-*N*-(α-methylbenzyl)amine **119**. In this case, the *N*-fluorobenzenesulfonimide was used as fluorinating agent with the aim to obtain α-fluoro-β^3-amino esters in up to quantitative yield and very high diastereomeric ratios [96] (Scheme **28**).

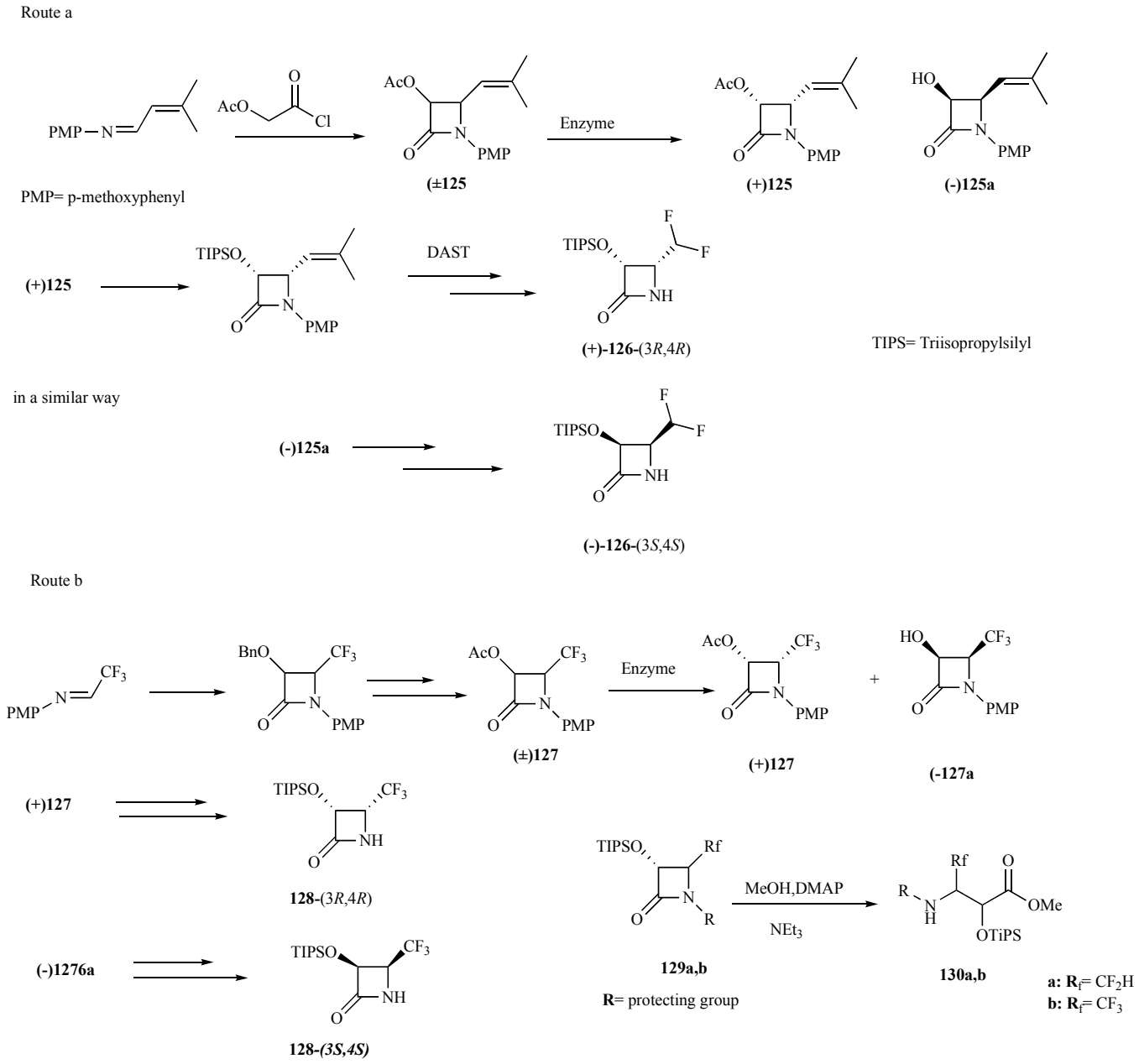


Scheme 28.

Because this methodology is not based on the use of α-amino acids from the chiral pool so, it potentially may allow the preparation of enantiopure α-fluoro-β^3-amino acids with a large variety of side chains. By this way, the synthesis of (2*S*,3*S*)-N^{β}-Fmoc-N^{ϵ}-Boc-α-fluoro-β^3-lysine **124** has been realized, starting from substrate **120** which was converted to enantiopure α-fluoro-β^3-amino ester **121**, as *anti* isomer. Then, a series of routinely steps, including conversion of the corresponding alcohol to the phthalimide **122** under Mitsunobu conditions, cleavage of the phthalimide and Boc protection, removal of the benzyl groups with the Pearlman's catalyst followed by Fmoc protection, gave **123** and, finally, cleavage of the *tert*-butyl ester and reprotection of the amine groups gave **124** (Scheme **28**). A novel approach for the synthesis of enantiopure fluorinated *N*-acyl-3-hydroxy-4-R_f-β-lactams (R_f = CF_2H, CF_3) has been successfully developed by means of [2 + 2] ketene-imine cycloaddition, followed by enzymatic optical resolution. After a simple methanolysis of fluorinated enantiopure β-lactams, the corresponding R_f-containing α-hydroxy-β-amino acid methyl esters were obtained in good to quantitative yields [97]. In details, through [2 + 2] ketene-imine cycloaddition, *cis*-1-PMP-3-AcO-4-(2-methyl-1-propenyl)-azetidin-2-one **125** was first synthesized in racemic form and then subjected to enzymatic optical resolution to afford β-lactams (3*R*,4*S*)-**125** and (3*S*,4*R*)-**125a**, with high enantiopurity (Scheme **29**, route a) [98].

The difluoromethylated azetidin-2-one **126** as (3*R*,4*R*)- and (3*S*,4*S*)- pure isomers, were obtained by the use of DAST as fluorinating reagent. For the preparation of 4-Tfm-β-lactams, a different strategy was employed: in this case the R_f moiety was introduced at the imine stage, before cycloaddition. Thus, after the key [2 + 2] ketene-imine cycloaddition step, the racemic *cis*-**127** was obtained, already containing the Tfm residue in the 4-position of β-lactam ring. The successive enzymatic resolution afforded **127** and **127a.** These compounds, in turn, were converted to corresponding (3*R*,4*R*)-**128** and (3*S*,4*S*)-**128** as described for **126** (Scheme **29**, route b). Enantiopure α-hydroxy-β-amino acid methyl esters bearing a CF_2H group or a CF_3 group at the C-3 position, **130a** or **130b**, were readily synthesized through a methanolysis of *N*-acyl-3-TIPSO-4-R_f-β-lactams, **129a** or **129b**, in the presence of triethylamine and catalytic amount of 4-(*N*,*N*-dimethylamino)pyridine (DMAP). Very recently, racemic β-trifluoromethyl-, β-difluoromethyl- and perfluoroethyl- substituted α- and β-amino nitrile derivatives were synthesized by means of regioselective 1,2-addition of trimethylsilyl cyanide (or metallated acetonitrile) to fluoroalkylated α,β-unsaturated imines. These fluorinated amino nitriles act as interesting starting materials for the preparation of new fluorinated diamines, fluoroalkylated amino acids and fluorine containing peptidomimetics [99].

In detail, trimethylsilylcyanide in methanol was added over the fluorinated imines **131** (R_F = CF_3, CHF_2, C_2F_5) to afford fluoroalkylated α-amino nitriles **132** in good yields keeping the *trans* configuration. Formation of fluoroalkylated α-amino nitriles **133**, involves an exclusive regioselective 1,2-addition of cyanide to α,β-unsaturated imines (Scheme **30**). After a basic hydrolysis of α-amino nitriles **132**, the corresponding fluorinated α-Tfm-α-amino

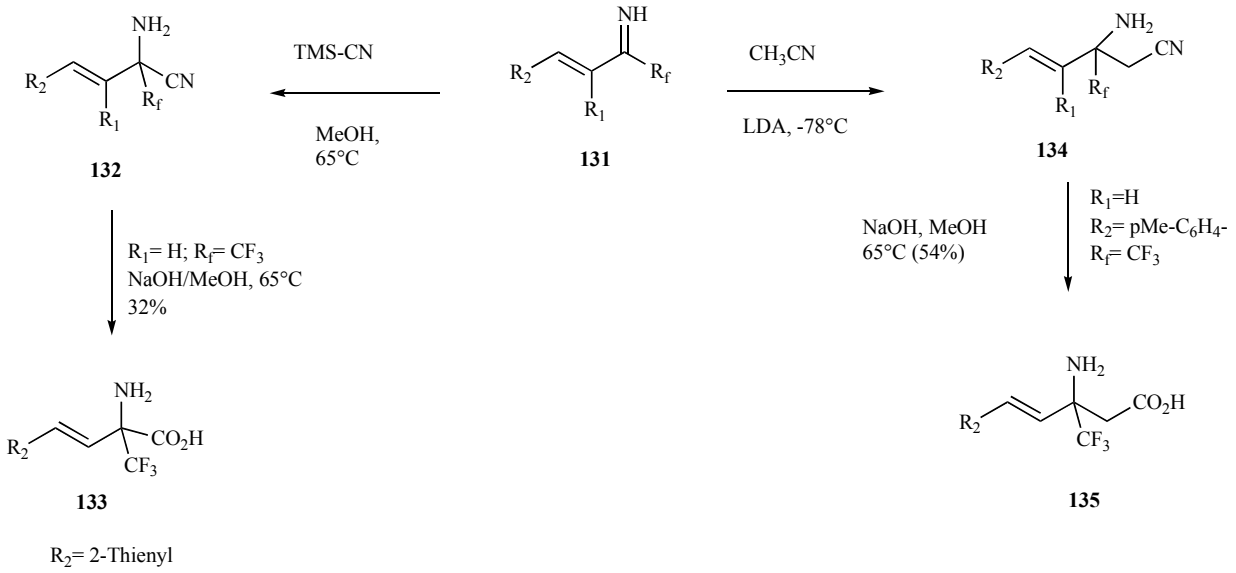


Scheme 29.

acid **133** was obtained in low yield. Differently, the selective 1,2-addition of the C-α carbanion derived from acetonitrile at −78 °C, over azadienes **131** gave, in a regioselective fashion, trifluoromethylated β-amino nitrile **134**. Compound **134** afforded the corresponding β-Tfm-β-amino acid **135** in moderate yield, after basic hydrolysis (Scheme **30**).

Fluorinated Amino Acids for Radiopharmaceutical Applications

Positron Emission Tomography (PET) is a high-resolution, sensitive, functional imaging technique, which can efficiently give access to the distribution, pharmaco-kinetics and -dynamics of a drug *in vivo*; so, it can therefore advantageously play a key-role in both drug discovery and development. This technique requires the preparation of a positron-emitting radiolabeled probe or radiotracer and for this purpose, fluorine-18 (^{18}F) is becoming, more and more often, the radionuclide of choice (adequate physical and nuclear characteristics and potential wide use and distribution of fluorine-18-labelled radiopharmaceuticals). During last decade, several amino acids have been labeled with either gamma radiation-emitting radionuclides or positron-emitting radionuclides, the most commonly used being ^{11}C. However, the longer half-life of ^{18}F (109 min, *vs* 20.5 min for ^{11}C) matches better with the relatively slow process of protein synthesis and also facilitates shipping of the radiolabelled amino acids to hospitals without an on-site cyclotron or dedicated radiochemistry laboratory. Moreover, the development of a variety of prosthetic groups has facilitated the efficient and site-specific labeling of peptides



Scheme 30.

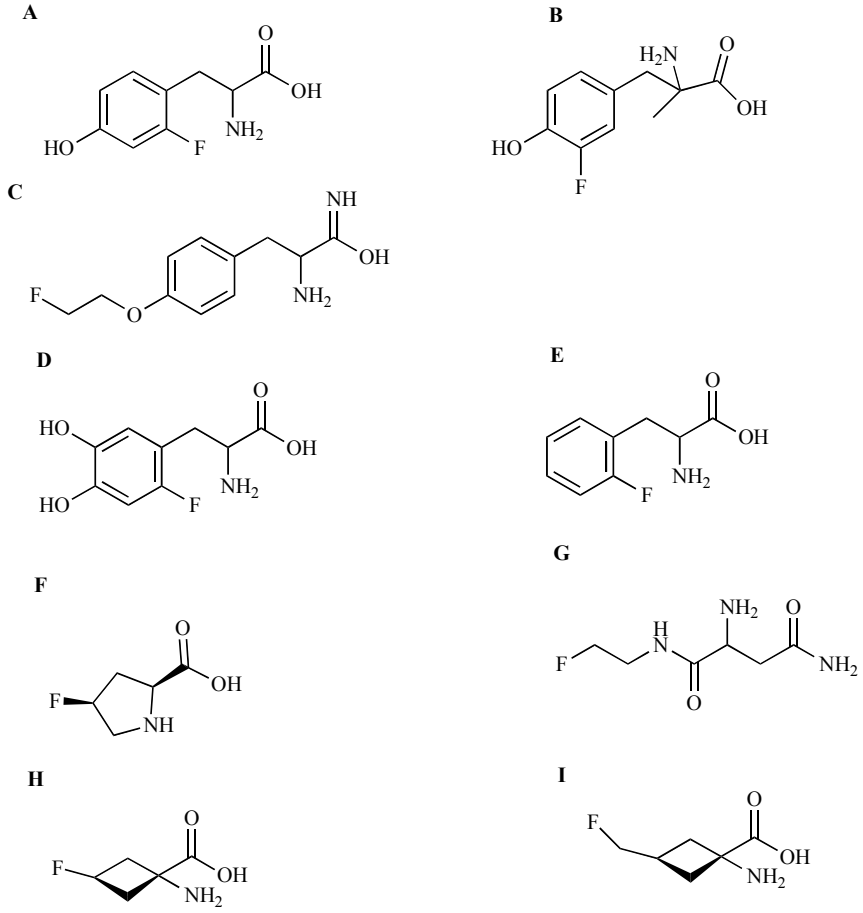
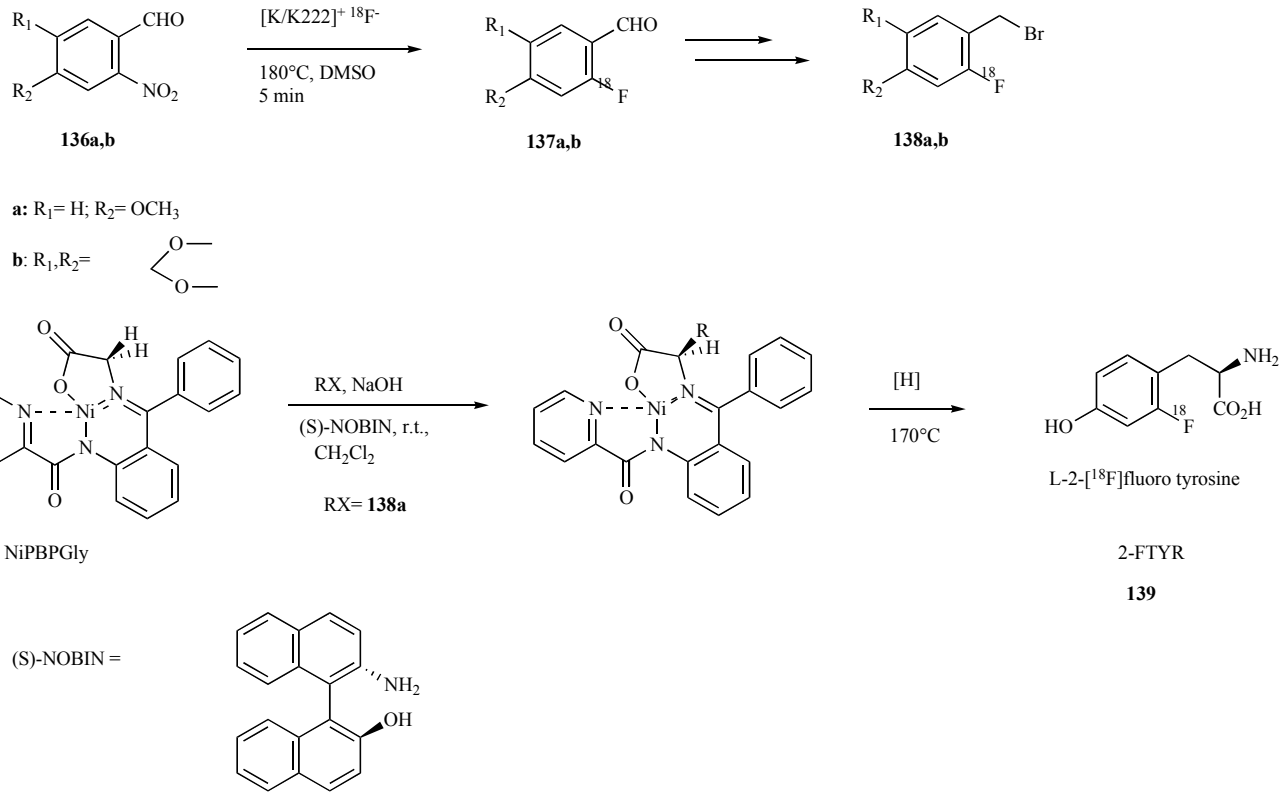


Fig. (2).

with ^{18}F. The ^{18}F-labeled peptides hold enormous clinical potential owing to their ability to quantitatively detect and characterize a wide variety of human diseases when using PET. A discrete number of ^{18}F-labeled bioactive peptides have shown great promise as diagnostic imaging agents [100]. While one of the best established amino acid for use in PET is ^{11}C-labeled methionine, the number of fluorinated amino acids under investigation is increasing, the most



Scheme 31.

widely studied being derivatives of tyrosine, along with phenylalanine and proline derivatives; tyrosine was labeled as L-2-[^{18}F]fluorotyrosine (**A**, 2-FTYR), as L-3-[^{18}F]fluoro-α-methyl tyrosine (**B**), and as *O*-(2-[^{18}F]fluoroethyl)-L-tyrosine (**C**) (Fig. **2**) [101].

A valid method for the asymmetric synthesis of [^{18}F]fluorinated aromatic α-amino acids, under phase transfer conditions (PTC), based on the use of electrophilic [^{18}F]fluorobenzyl bromide derivatives in the stereoselective alkylation of Ni(II) complex of a Schiff base of 2-benzoylphenylamide of pyridine-2-carboxylic acid (PBP) and glycine (NiPBPGly) in the presence of 2-amino-2'-hydroxy-1,1'-binaphtyl [*(R)*- or *(S)*-NOBIN], as an original substrate/catalyst pair, has been published (Scheme **31**) [102].

In details, after the initial preparation of the 2-[^{18}F]fluorobenzyl bromide derivatives **138a** and **138b**, based on a classical nucleophilic substitution on a nitro leaving group of 2-nitrobenzaldehyde derivatives **136a** and **136b** using a reactive [K/K2.2.2]$^{+18}$F^{-} complex, the key alkylation step on NiPBPGly Nickel complex proceeded under mild conditions, allowing the preparation of (2-FTYR) **139** (Scheme **31**) and L-6-[^{18}F]fluoro-3,4-dihydroxyphenyl-alanine (**D**, 6-FDOPA, Fig. **2**), with an e.e. of 92% and 96%, respectively. A similar strategy, that is exploiting the same nucleophilic substitution pathway, starting from an appropriate tosylate precursor, based on no-carrier-added (NCA) [^{18}F]fluoride, which is available in large amounts from proton irradiation of ^{18}O-enriched water, afforded ^{18}F-labeled branched α-amino acid, namely 2-amino-4-[^{18}F]fluoro-2-methylbutanoic acid (FAMB) [103a,b].

Technetium labeling procedures have also been used for radiopharmaceutical applications. In fact, the radiolabeled peptides with ^{99m}Tc offer the possibility of a wide array of compounds for variety of applications in diagnostic and therapeutic medium [104].

CONCLUSIONS

Different synthetic procedures have been developed to modify the amino acid side-chain and backbone of peptides. The oxidative approach is mainly focused on transformation in side-chain of low redox value residues, while fluorination is operative on both side-chain and backbone moieties. The possibility to modify preformed amino acids or peptides, connected with the possibility to produce modified proteins by genetic engineering open new strategies for the synthesis of high active and low toxic unnatural peptides and proteins.

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