Fluorine Containing Molecules for Peptidomimicry: A Chemical Act to Modulate Enzymatic Activity

C.E. Oyiliagu[1], M. Novalen[1] and L.P. Kotra[1,2,*]

[1]*Molecular Design and Information Technology Center, Leslie Dan Faculty of Pharmacy, University of Toronto, Toronto, ON M5S 2S2 Canada,* [2]*Division of Cell and Molecular Biology, Toronto General Research Institute, University Health Network, 150 Gerrard Street West, Toronto, Ontario, M5G 2C4, Canada*

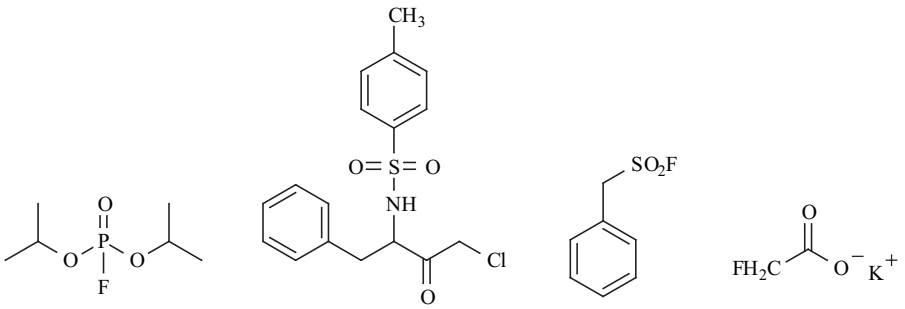
Abstract: Fluorine atom has been used extensively to modulate the properties of various peptide-like compounds to modulate enzyme activities such as proteases. Fluorinated functionalities such as trifluoromethyl group, difluoromethyl group, fluoromethyl moiety and recently -CHF-S- were investigated. This article discusses important fluorine containing peptidomimetics, synthetic strategies, and the modulation of enzymic activities.

Keywords: Fluoropeptidomimetics, fluorine substitution, peptidomimicry, protease inhibitors.

INTRODUCTION

Chemists design and develop novel core structures and analogs carrying a number of elements. In organic chemistry, although the number of such elements is limited to a handful in the periodic table, we are still discovering novel properties attributed to various combinations of elements in organic molecules. Fluorine is an interesting element that attracted the attention of the organic chemists almost six decades ago in relation to biological activity modulation. Fluorine was introduced as an element of "curiosity" into a number of biologically active molecules, including peptidomimetics. Peptidomimicry is a strategy to replace key portions of peptides with more stable non-peptidic ("amide") structures. Several peptidomimetics act as inhibitors of proteases, lactamases and esterases, among others. Typically the scissile peptide bond of the substrate is replaced by a non-hydrolyzable, but a structurally similar motif to create a peptidomimetic compound. Examples include transition-state mimics, substrate analogs on a peptide template, replacement of the peptide backbone by conformationally-restricted non-peptidic structures and other variations mimicking the structures of peptides [1-3].

Irreversible inhibition of serine proteases, for example, is usually mediated using substrate analogs containing electrophilic residues that form covalent bonds with serine or histidine in the catalytic triad. Examples of such residues include alkyl fluorophosphates (**1**), chloromethyl ketones (**2**) and sulfonyl fluorides (**3**) (Fig. **1**) [2,4]. However, with irreversible inhibitors, there exists the possibility of reactions between the inhibitor and other nucleophiles present in the human body if such inhibitors are not specific and are used as drugs [2]. Most inhibitors of aspartyl proteases are reversible in their mode of action. However, where there is a paucity of electrophilic isosteres from which potent and selective reversible inhibitors can be developed, as with cysteine proteases, the focus is turned towards optimizing alkylating functional groups [2].



1 **2** **3** **4**

Fig. (1). Structures of the irreversible serine protease inhibitors **1-3** and the potassium fluoroacetate toxin **4** from the South African gifblaar shrub.

The incorporation of a fluorine atom into biologically active molecules stemmed from such discoveries as that of the potassium fluoroacetate toxin **4** of the South African gifblaar shrub (*Dichapetalum cymosum*) by J. S. Marias in 1943, one of the early findings that generated vast interest in the effect of fluorine substitution (Fig. **1**) [5-7]. Following the demonstration by Fried and Sabo in 1954 of the improved potency of 9α-fluorohydrocortisone compared to

*Address correspondence to this author at the Division of Cell and Molecular Biology, Toronto General Research Institute, University Health Network, 150 Gerrard Street West, Toronto, Ontario, M5G 2C4, Canada; Tel. (416) 978-2882; Fax. (416) 978-8511; E-mail: p.kotra@utoronto.ca

1570-193X/06 $50.00+.00

cortisol acetate, an appreciation for the potential of fluorine atom to dramatically improve the pharmacodynamic and pharmacokinetic properties of drug candidates was developed [6,7,8].

The modulating properties of the fluorine atom are thought to arise from its unique properties such as the high Pauling electronegativity value of 3.98, its relative small size, low polarizability and tightly bound non-bonding electron pairs [9]. As a result, the electronic, lipophilic, steric characteristics, metabolic stability and solubility can be simultaneously altered by introducing fluorine onto a compound [10]. Electronic structure changes as a result of fluorine substitutions may lead to the modulation of drug-receptor interaction by introducing additional hydrogen bonding potential while improving a drug's lipophilicity [7,11]. Furthermore, the small size of fluorine, with a van der Waals radius of 1.47 Å falling in between that of oxygen (1.52 Å) and hydrogen (1.20 Å), enables fluorine to mimic a hydroxyl group in some molecules or a hydrogen atom in others without creating any major steric disturbance in the compound [6,7,9].

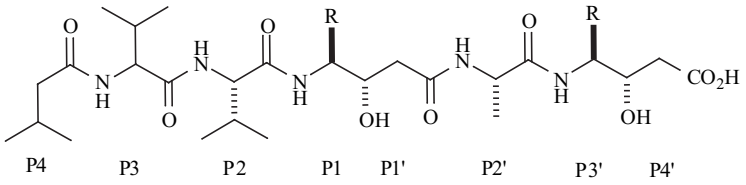
This strategy of drug discovery by the ingenious introduction of fluorine has been implemented in the design of a variety of protease inhibitors. Among these novel approaches are α-trifluoromethyl malic hydroxamate analogs, and partially modified retro- and retro-inverso Ψ[NHCH(CF_3)]Gly peptides as inhibitors of matrix metalloproteases, difluorostatine and difluorostatone analogs as inhibitors of aspartyl and serine proteases, as well as fluoropeptidomimetics carrying the key moiety "-CHF-S-" as inhibitors of serine proteases. These and other approaches in the context of mono, di- and tri-fluoro substitutions, inhibition strategies and chemical syntheses are discussed in this article.

1. THE CF_3 MOIETY

The trifluoromethyl group (CF_3) is frequently used in medicinal chemistry as a mimic for various other functional groups such as methyl, isopropyl and phenyl, and can be found in a number of biologically active and therapeutic agents [7,12]. It is hydrophobic, electron-rich and bulky, and sometimes it is also considered to have xenobiotic characteristics. The trifluoromethyl group enhances the *in vivo* stability of a compound because removal or substitution of a fluorine atom in the trifluoromethyl group (as opposed to its methyl isostere) cannot be done by the metabolic enzymes [13]. Such biological effects could also be very attractive in drug design to alter the transport of the drug to the site of action, such as a tumor site, brain or a site of infection [14]. In fact, CF_3 can be found in various drugs that penetrate the blood-brain barrier. As an example, many of the dopamine receptor-blocking neuroleptics in the central nervous system, such as tricyclins, buterophenones and diarylbutylamines contain either the CF_3 or fluoro-phenyl group. These groups help improve the pharmacological activities of the drugs by enhancing transport across the blood-brain barrier and by decreasing degradation [14].

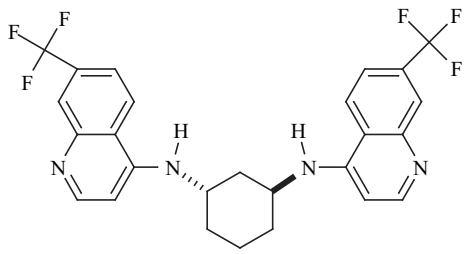
Extensive work has been carried out by Zanda in studying the effect of incorporation of the trifluoromethyl group into peptidomimetics [12]. Trifluoromethyl moiety was incorporated into Pepstatin A, a subnanomolar inhibitor of many aspartyl proteases by replacing the isobutyl group to produce compound **5** (Fig. **2**) [12]. The substitution was performed to investigate the biological activity of the fluorinated inhibitor against several aspartyl proteases including HIV-protease, a protease for which pepstatin A has no affinity. The modified inhibitor, *bis*-trifluoromethyl pepstatin **5** did not show any effect on proteolytic activity of HIV-protease at concentrations up to 150 μM, but a much stronger activity was found towards plasmepsin II, an aspartic protease of the malaria-causing protozoa, *Plasmodium falciparum* [12,15]. X-ray crystallographic analyses of pepstatin A and the fluorinated analog **5** bound to plasmepsin II showed a very similar conformation for both compounds demonstrating that the trifluoromethyl group can be successfully used to mimic an isobutyl moiety [12,15]. Interestingly, incorporation of the trifluoromethyl group into the *bis*-quinoline antimalarial drug **6** (Roche Pharmaceuticals) decreased the antimalarial activity compared to the non-fluorinated lead compound possibly due to sterically disfavored drug-receptor interactions (Fig. **3**). Similar effect was also observed for trifluoromethyl-containing analogs of metaquine, another antimalarial drug [17]. However, there are also a number of trifluoromethyl-substituted antimalarial drugs that are more potent than their non-fluorinated analogs indicating that the biological effects of such modifications vary from case to case [7].

Matrix metalloproteases (MMPs) are zinc (II)-containing proteolytic enzymes that are involved in the degradation of the extracellular matrix. Regulation of the activity of MMPs is a proven strategy for the treatment of several diseases including cancer and arthritis [17]. Compounds carrying a terminal hydroxamate group HONHC(O)- have dominated the chemical design because this functionality coordinates effectively with Zn^{2+} that is present in the active sites of



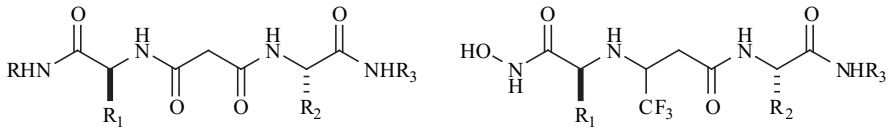
Pepstatin A, R = *iso*-butyl
5, R = CF_3

Fig. (2). Natural Pepstatin A and its bis-trifluoromethyl analog **5**.



6

Fig. (3). Structure of bis-quinoline trifluoromethyl analog **6**.

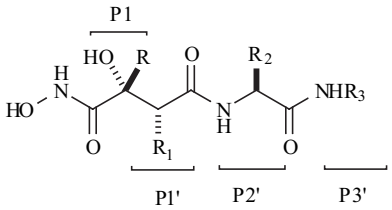


7 **8**

Fig. (4). Structures of the conventional malonate-based retro-Ψ[NHCO]-peptide **7** and retro-Ψ[NHCH(CF_3)]-peptide **8** hydroxamates.

these metalloproteases. One of this class of molecules, partially modified retro-Ψ[NHCH(CF_3)]-peptidyl hydroxamates (**8**), as a novel class of hydroxamates containing a -CH(CF_3)CH_2CO- unit, were synthesized as a substitute for the malonyl moiety of partially modified retropeptides such as **7** (Fig. **4**) [18,19]. Randomly chosen trifluoromethyl-retropeptidyl hydroxamates showed moderate to weak inhibitory activity, indicating that high-throughput screening of the novel hydroxamate libraries may yield reasonable hits [19]. Further research was concentrated on optimizing the synthetic approach and on generating analogs such as partially modified retro-inverso Ψ[NHCH(CF_3)]-peptidyl hydroxamates [20].

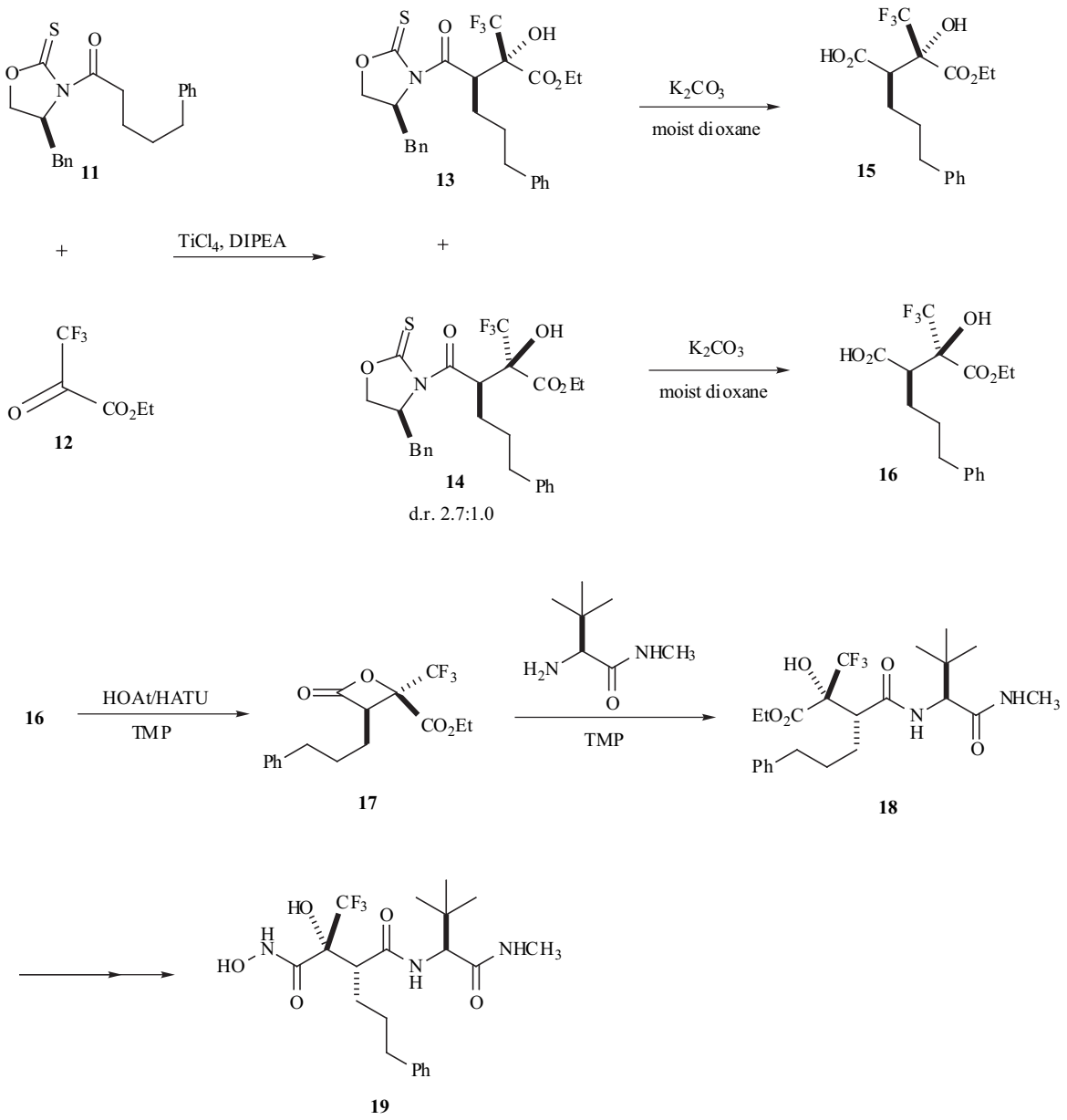
A few years ago, a new family of potent peptidomimetic hydroxamate inhibitors such as **9** against MMP-1, MMP-3 and MMP-9 was described by Jacobson and co-workers (Fig. **5**) [21,22]. These structures carried a quaternary-hydroxyl moiety at P1 and several different R substitutions at P1'. Zanda and co-workers incorporated fluoroalkyl susbstituents at the P1 quaternary position in **9** and synthesized molecules such as **10**, anticipating an enhancement in selectivity towards different MMPs by optimizing binding properties (Fig. **5**) [23]. The core α-trifluoromethyl malic unit was obtained from $TiCl_4$ catalyzed aldol reaction of *N*-acyl-oxazolidin-2-thione **11** with ethyl trifluoropyruvate **12** (Scheme **1**). This yielded two diastereomeric adducts **13** and **14** both of which were employed in the synthesis of the target compounds. The oxazolidin-2-thione was cleaved using K_2CO_3 in aqueous dioxane to yield the carboxylic acid derivatives **15** and **16** (Scheme **1**). It is interesting to note that the cleavage of **13** and **14** using the standard conditions of benzyl alcohol and catalytic amounts of DMAP required one week in refluxing dichloromethane and led to partial α-epimerization. Coupling of **15** using the HOAt/HATU system and saponification of the carboxylic ester bound to the quaternary α-trifluoromethyl carbinolic center occurred smoothly. However coupling of the resulting carboxylic acid to benzyloxyamine was unsuccessful using various conventional coupling agents for peptides including DCC/DMAP, EDC/HOBt, DIC/HOBt, HATU/HOAt, and PyBroP/DIPEA. This observation was attributed to its low reactivity and high steric hindrance [23]. Eventually, freshly prepared $BrPO(OEt)_2$ was found to be reasonably effective as a coupling agent for this adduct. With the diastereomeric adduct **16**, coupling using the HOAt/HATU generated the β-lactone **17** in addition to the expected coupling product **18**. β-Lactone **17** was then used for the generation of additional amounts of compound **18**. Saponification of **18** led to a partial epimerization of the stereo center carrying the phenylpropyl moiety. The epimers were separated after coupling with $BnONH_2$. Hydrogenolysis provided compound **19**, a diastereomerically pure trifluoromethyl analog of **9** with R_1 = -$(CH_2)_3$Ph, R_2 = *t*-Bu and R_3 = CH_3. While **19** exhibited the most potent inhibition against MMP-2 and MMP-9 of the target trifluoromethyl hydroxamates, it was less potent than its nanomolar CH_3 analog **9**, and exhibited little selectivity. Its lower activity was hypothesized to be due to the inability of the bulky, electron-rich CF_3 group to either assume the crucial binding conformation of its CH_3 analog or to fit into the S1 pocket [23].



9, R = CH_3
10, R = CF_3

Fig. (5). Structures of the inhibitor **9** and its CF_3 analogue **10.**

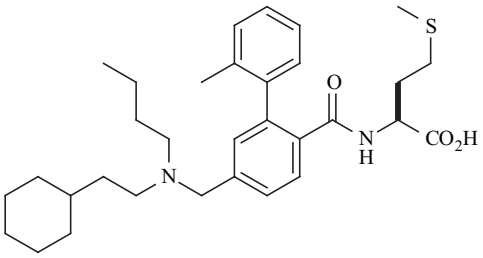
Another interesting enzyme, farnesyl transferase has been pursued as a target for inhibition for cancer treatment, and currently there are several compounds undergoing clinical trials [24]. Wang and co-workers discovered a lead compound ABT-839, a potent farnesyl transferase inhibitor with good selectivity and cellular activity, but lacking oral bioavailability, a property that was assumed to be due to its methionine moiety (Fig. **6**) [25]. In an attempt to generate potent inhibitors without methionine, computer modeling



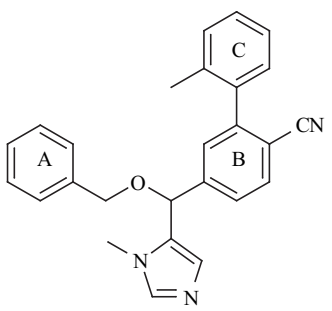
Scheme 1. [ref. 23].

was applied, leading to the design and synthesis of compound **20** as the lead structure featuring the signature *o*-tolyl biphenyl core of ABT-839, but carrying a cyano group on the B ring instead of the acyl methionine moiety (Fig. **6**). Compound **20** had modest inhibition and selectivity properties; therefore different optimization strategies were applied [26]. A library of 80 analogs containing either ethereal heterocycles or benzyl rings with *ortho*, *meta* or *para*-substitution showed that electron-withdrawing substitutions generated more potent inhibitors compared to the electron-donating ones. A *p*-cyano benzyl substitution on the A ring was found to yield the most active compound. For optimization of the C ring, *ortho*-trifluoromethyl substitution (compare ring C in **20** *vs.* **21**) stood out as the most attractive inhibitor, greatly enhancing farnesyltransferase inhibition, cellular activity and selectivity [26]. Further optimization led to the generation of a final set of compounds **21** bearing a 2-amino-3-cyano-6-pyridyl ring A and an *ortho*-trifluoromethyl substituted ring C (Fig. **6**).

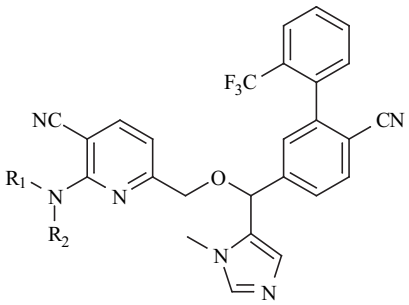
Compounds with an aliphatic cyclic amino substitution (R_1 and R_2) were the most potent analogs, and were constructed from the appropriate aniline precursors as shown in Scheme **2**. Treatment of aniline ester **22** with molecular bromine afforded the bromo aniline ester **23**, which was converted to a cyano bromoaldehyde *via* the diazonium salt formed from the amine followed by DIBAL reduction of the ester. The reaction of cyano bromoaldehyde **24** with *o*-trifluoromethylbenzene boronic acid produced compound **25**, which was then treated with the lithium salt of 1-methyl-2-triethylsilyl imidazole to give alcohol **26**. Silver oxide catalyzed the alkylation of **26** with 2-chloro-3-cyano-6-bromomethyl pyridine produced the key intermediate **27,** which upon treatment with appropriate amines afforded compounds of type **21** [26].



ABT-839

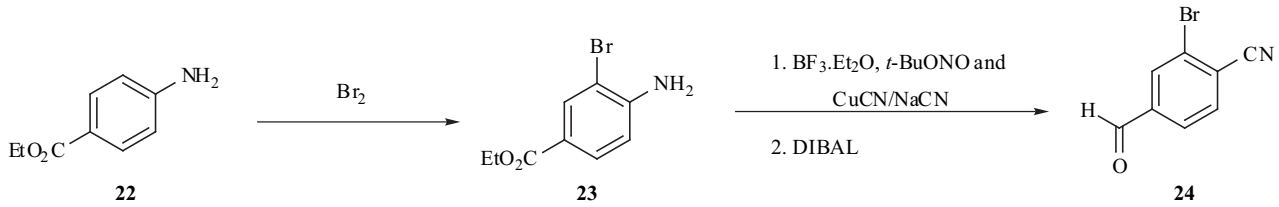


20

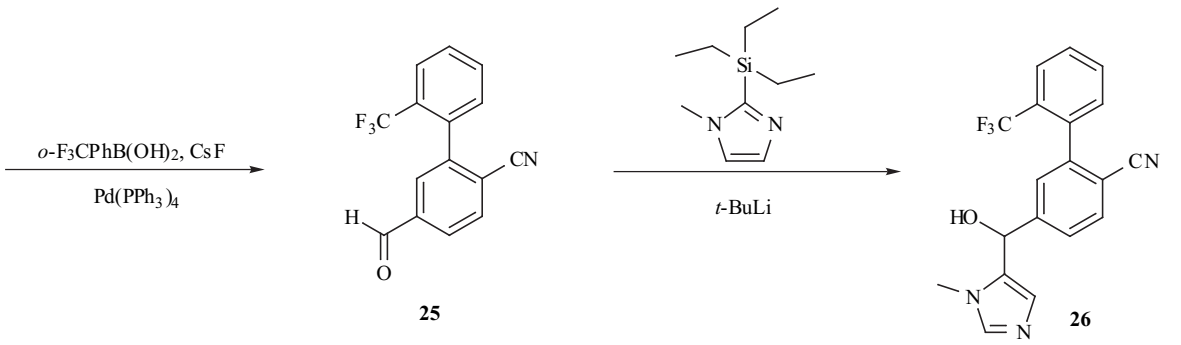


21

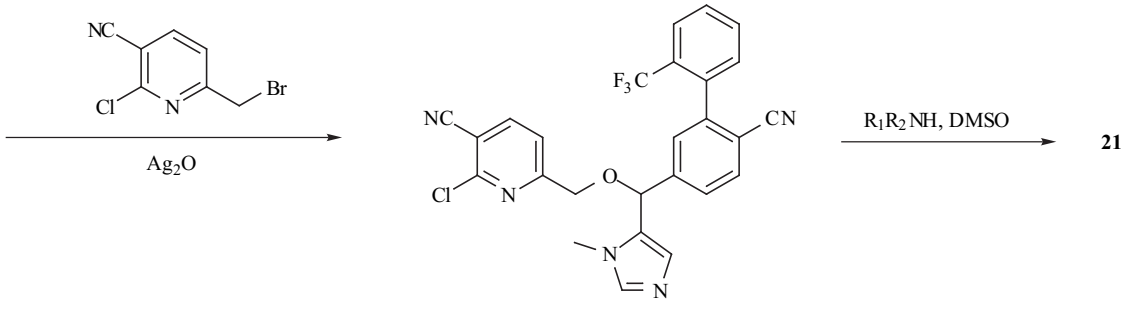
Fig. (6). The first generation clinical candidate ABT-839, the lead compound **20**, and compound **21** from the optimization of **20**.



22 **23** **24**



25 **26**



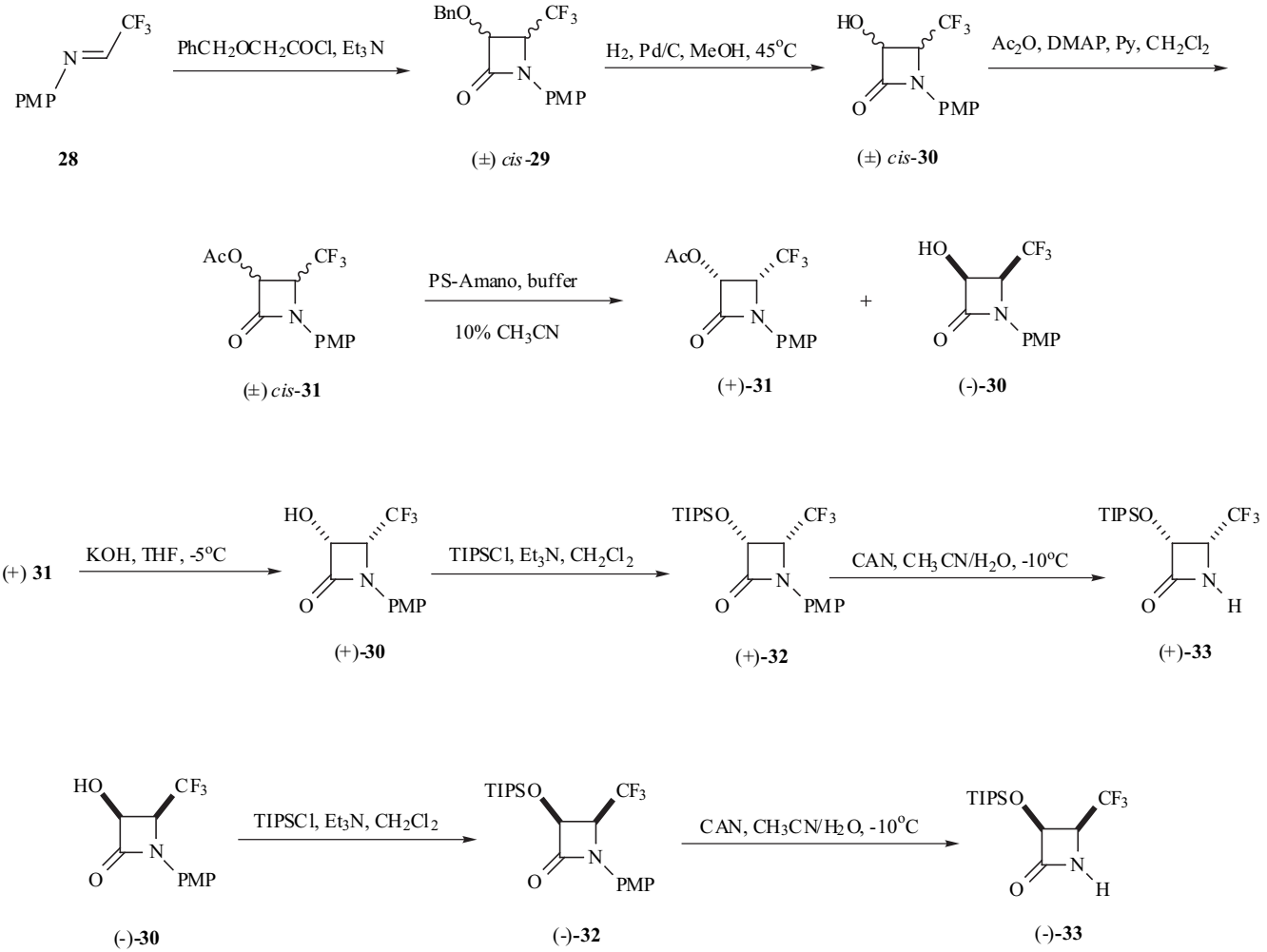
27

Scheme 2. [ref. 26].

Fluorinated Amino Acids, Dipeptides and Fluoro Taxoids

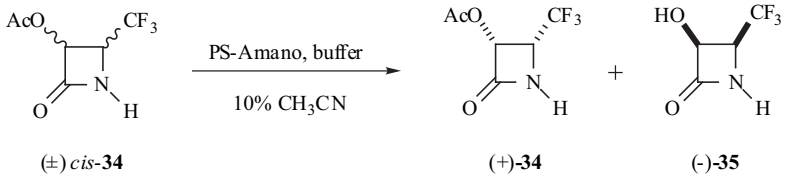
α-Hydroxy-β-amino acids are well represented in a large number of biologically active compounds such as antitumor agents, immunological response modifiers and HIV protease inhibitors. Due to the improved pharmacological properties that often follow the incorporation of fluorine into a molecule, interest arose for the synthesis of fluorine-containing α-hydroxy-β-amino acids that can serve as important bioactive compounds for medicinal chemistry and chemical biology [27].

A new synthetic approach to fluorine-containing α-hydroxy-β-amino acids and their congeners from enantiopure trifluoromethyl-containing β-lactams was developed by Kuznetsova and co-workers using the β-lactam synthon method [27]. In this method, the strained four-membered β-lactam is highly activated towards nucleophilic attack by acylation of its nitrogen, allowing for facile ring-opening reactions. First, a practical method for the synthesis of enantiopure 3-hydroxy-4-trifluoromethyl-β-lactams was developed (Scheme **3**). *N*-*p*-Methoxyphenyl trifluoroacetaldimine (**28**) was subjected to a [2+2] ketene-imine cycloaddition using benzyloxyacetyl chloride as the ketene precursor to produce racemic *cis*-3-benzyloxy-4-trifluoromethyl-β-lactam, (±)-**29** in a moderate yield. The benzyl protecting group was then converted to an acetyl group by hydrogenolysis followed by acetylation to yield racemic *cis*-3-acetoxy-4-trifluoromethyl-β-lactam (±)-**31**, which was subjected to enzymatic optical resolution using commercially available PS-Amano lipase, generating optically pure (3*R*,4*R*)-3-acetoxy-4-trifluoromethyl-β-lactam (+)-**31** and (3*S*,4*S*)-3-hydroxy-4-trifluoromethyl-β-lactam (-)-**30**. Hydroxyl moiety was finally protected using triisopropylsilyl group in (-)-**30** and (+)-**31** followed by the removal of *N*-*p*-methoxyphenyl group using cerium



28 (±) *cis*-**29** (±) *cis*-**30** (±) *cis*-**31** (+)-**31** (-)-**30** (+) **31** (+)-**30** (+)-**32** (+)-**33** (-)-**30** (-)-**32** (-)-**33**

Scheme 3. [ref. 27].



(±) *cis*-**34** (+)-**34** (-)-**35**

Scheme 4. [ref. 27].

ammonium nitrate to produce the precursors (+)-**33** and (-)-**33** for *N*-acylation (Scheme **3**). An alternative route to the generation of 4-trifluoro-β-lactams involved the removal of *N*-protecting *p*-methoxyphenyl moiety from the racemic *cis*-3-acetoxy-4-trifluoromethyl-β-lactam (±)-**34** prior to enzymatic optical resolution (Scheme **4**). The optical resolution of the compound (±)-**34** with a free amino moiety was found to occur much faster than *N*-*p*-methoxyphenyl-β-lactam, (±)-**31** and both enatiomerically pure products were found to be stable at 25 ºC unlike (-)-**30** [27].

It should be noted that the β-lactams containing CF_3 were synthesized using a different approach (*vide supra*) compared to 3-hydroxy-4-difluoromethyl-β-lactams. For the trifluoromethyl β-lactams, the presence of CF_3 prior to the [2+2] cycloaddition led to a number of necessary alterations to the experimental procedure (Scheme **3**). Benzyloxyacetyl chloride was used to generate the ketene for the reaction with CF_3-imine in place of acetoxyacetyl chloride which was found to be unreactive. Furthermore, the reduced nucleophilicity of the nitrogen in compound **28** required a higher temperature (40 ºC) before the reaction could proceed satisfactorily. Treatment of the racemic 3-acetoxy-4-trifluoromethyl-β-lactam, (±)-**31** with PS-Amano lipase pH 7 led to the isolation of only (+)-**31** probably due to the further hydrolysis of the β-lactam ring of (-)-**30** [27].

Fig. **7** highlights representative examples of transformations of the fluorinated β-lactam derivatives. *N*-Acylation of the resulting β-lactams followed by hydrolysis led to the generation of fluorinated α-hydroxy-β-amino acid **36** while the application of facile methanolysis of these compounds produced the corresponding trifluoromethyl-containing α-hydroxy-β-amino acid methyl esters **37** in good to quantitative yields. Corresponding trifluoromethyl dipeptides **38** – **39** and the taxoids **40** were obtained in good yields by coupling β-lactams with amino acid esters and baccatins, respectively [27].

2. THE CF_2 MOIETY

The CF_2 moiety has been used for the introduction of additional P' substituents for interaction with the S' subsites in proteases. Such peptidomimetics have been employed as inhibitors of a range of proteases including aspartyl and serine proteases. Such applications of fluorine in peptidomimicry include difluorostatine analogs, difluorostatone analogs and difluoromethylketone isosteres.

Following are a few illustrative examples of each case where the scissile amide bond of the native peptide substrate **41** (Fig. **8**) is replaced by various groups. Investigation into the inhibitors of renin, an aspartyl protease, generated the N-terminal, statine-modified, angiotensinogen derivative **42** (Fig. **9**) [28]. Statine was proposed to mimic the tetrahedral intermediate formed during peptide hydrolysis [29]. Initial attempts to mimic the statine moiety was carried out by oxidizing the hydroxyl group of statine in order to generate a carbonyl analog that would be converted to a geminal diol

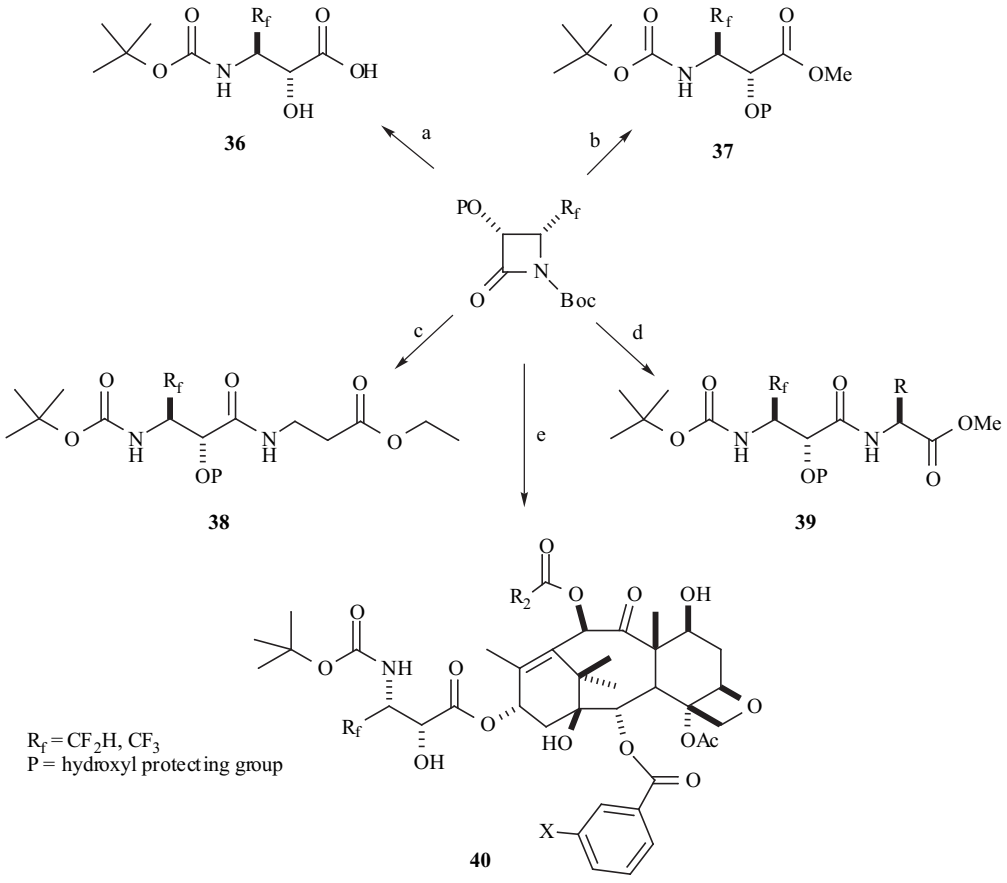
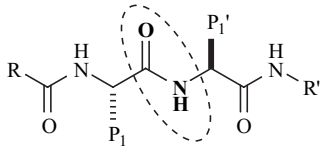


Fig. (7). Representative examples of transformations of *N*-*t*-Boc-β-lactam derivatives: a) hydrolysis, b) methanolysis, c) coupling with β-amino ester, d) coupling with α-amino ester, e) ring-opening coupling with baccatin.

within the active site of the aspartyl protease by hydration, thereby mimicking the transition state of peptide hydrolysis by aspartyl proteases. However, the resulting inhibitor was less potent than the corresponding statine-containing peptide [30].



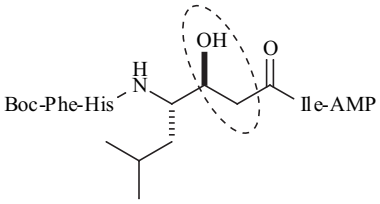
41

Fig. (8). A general structure of the native peptide substrate of proteases.

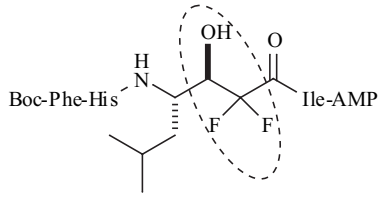
By postulating that the reduced inhibition of the carbonyl analog was due to its sluggishness towards hydration, Thaisrivongs and co-workers investigated the effect of introducing electron-withdrawing fluorine atoms to the unsubstituted methylene group *alpha* to the ketone [28]. The electron-withdrawing nature of fluorine was proposed to activate the carbonyl ketone towards nucleophilic attack, facilitating hydration [28]. A series of compounds was synthesized including **43** and **44** in addition to a diastereomer of **43** at the hydroxyl position, an epimer of **44** at the leucine side chain (P1), and analogs with benzyl and cyclohexylmethyl groups at P1. Synthesis of the key intermediates was achieved by Swern oxidation of the Boc-protected amino alcohol to an aldehyde followed by treatment with ethyl bromodifluoroacetate and activated Zn (Reformatsky conditions) to obtain a separable diastereomeric mixture. These diastereomers were subjected to standard ester hydrolysis, carbamate-deprotection and peptide coupling to obtain the target compounds [28]. While the 3(*S*)-hydroxyl difluoro analog **43** showed an IC_{50} of 12 nM and the statine analog **42** with an IC_{50} of 1.7 nM, its 3(*R*)-hydroxyl diastereomer of **43** showed an IC_{50} of 730 nM. One explanation of the lower inhibition of the difluorostatine moiety **43** compared to **42** was the importance of the hydroxyl group as a hydrogen-bond acceptor, a property that fluorine interferes with by reducing the electron density on the hydroxyl in compound **43** [28]. Compound **44** with difluorostatone in place of statine was found to be more potent than either **42** or **43**, with an IC_{50} of 0.52 nM. Attempts were made to optimize the inhibition by substituting leucine with the more lipophilic side chains cyclohexyl methyl and phenylalanine. However, such compounds showed comparable inhibition to those by compounds **42-44**. Compound **44** (with racemized L-Lue) also exhibited 3 to 4 orders of magnitude higher selectivity against human plasma renin compared to porcine pepsin and bovine cathepsin [28].

Eda *et al.* in 1998 published the synthesis and biological evaluation of difluoromethylene ketone derivatives **45** against human heart kinase, a chymotrypsin-like serine protease (Fig. **10**) [31]. In agreement with their hypothesis, it was found that difluoromethylene ketone derivatives were more potent and selective inhibitors of human heart kinase over bovine α-chymotrypsin, a closely related serine protease, when compared to the trifluoromethyl ketone **46**. Compound **46** exhibited a moderate inhibition against human heart kinase, and little selectivity over bovine α-chymotrypsin. Its inhibition was based on favorable interactions between Val-Pro-Phe and the S subsites of human heart kinase as well as transition state mimicry. Selectivity over bovine α-chymotrypsin and increased affinity for human heart kinase were achieved by synthesizing difluoro analogs **45** with a P' residue. An S'/P' interaction was hypothesized to improve the inhibitor's potency based on a proposal by Kinoshita. This linked the high substrate specificity of human heart kinase for angiotensin-I to the unique conformation of its S' subsites [32].

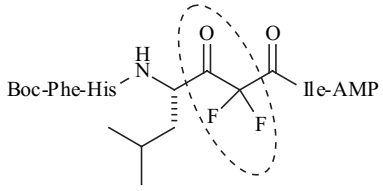
The most potent difluoromethylene ketone analogs carried either phenyl or carboxylic acid groups at P' oriented in the proper position and direction. An inhibition constant (K_i) of 1.3 ± 0.4 nM was observed for difluoromethylene ketone mimic **47** (Fig. **10**). Generally, the difluoromethylene ketone analogs showed an improved selectivity for human heart kinase over bovine α-chymotrypsin, some exhibiting a selectivity ratio between bovine α-chymotrypsin (K_i)/



42

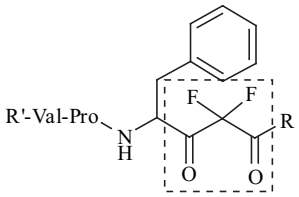


43

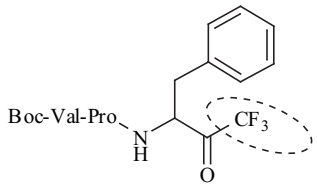


44

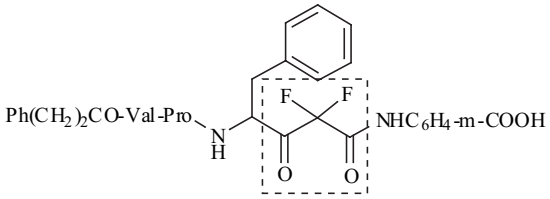
Fig. (9). Analogous inhibitors of human plasma renin containing the statine moiety **42**, the difluorostatine moiety **43**, and the difluorinated ketone (or statone) moiety **44**.



45



46



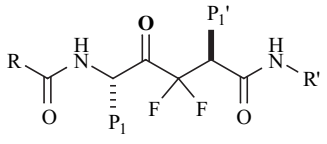
47

Fig. (10). Difluoromethylene ketones and trifluoromethyl ketones employed in the inhibition of human heart kinase.

human heart kinase (K_i) of 150 times compared to that of trifluoromethyl ketone **46** [31].

The aforementioned illustrations describe the use of difluorostatine and difluorostatone containing peptidomimetics as potent inhibitors of aspartyl and serine proteases. Although these inhibitors lack a P1' group, their relative ease of synthesis and stability make them more attractive than true dipeptide isosteres. Inhibitors of the general structure **48** are encountered far less frequently in the literature (Fig. **11**) [33,34]. Reasons for the rarity of this type of protease inhibitor include a lack of readily available and widely applicable synthetic procedures as well as the potential reactivity of the intermediates and final compounds (Scheme **5**). Compound **49** may be formed by HF elimination in the presence of a base, and compound **50** by spontaneous cyclization (Scheme **5**) [33,34].

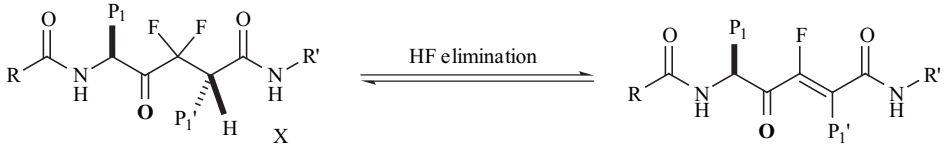
Damon and Hoover developed an elegant pathway to synthesize ketodifluoromethylene dipeptide isosteres (Scheme **6**) [35]. (*R*)-Phenylacetic acid **51** was esterified by acid catalysis to give compound **52** followed by a catalytic reduction (Rh-C) to yield a cyclohexylmethyl derivative **53**



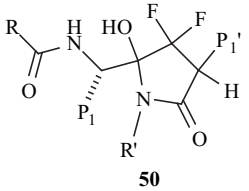
48

Fig. (11). A difluoro ketomethylene dipeptide isostere.

in 92% yield. *O*-Silyl protection of compound **53** followed by DIBAL reduction of the methyl ester led to the aldehyde **55** in an excellent yield. Reaction of **55** with dibromodifluoromethane yielded the difluorinated allylic ether **56** introducing the difluoro moiety into the template. Desilylation, yielding **57**, followed by esterification by sequential treatment with *n*-BuLi and 4-methylvaleryl chloride gave compound **58** in 84% yield. Enolization of **58** using $LiN(TMS)_2$ followed by the sequential addition of

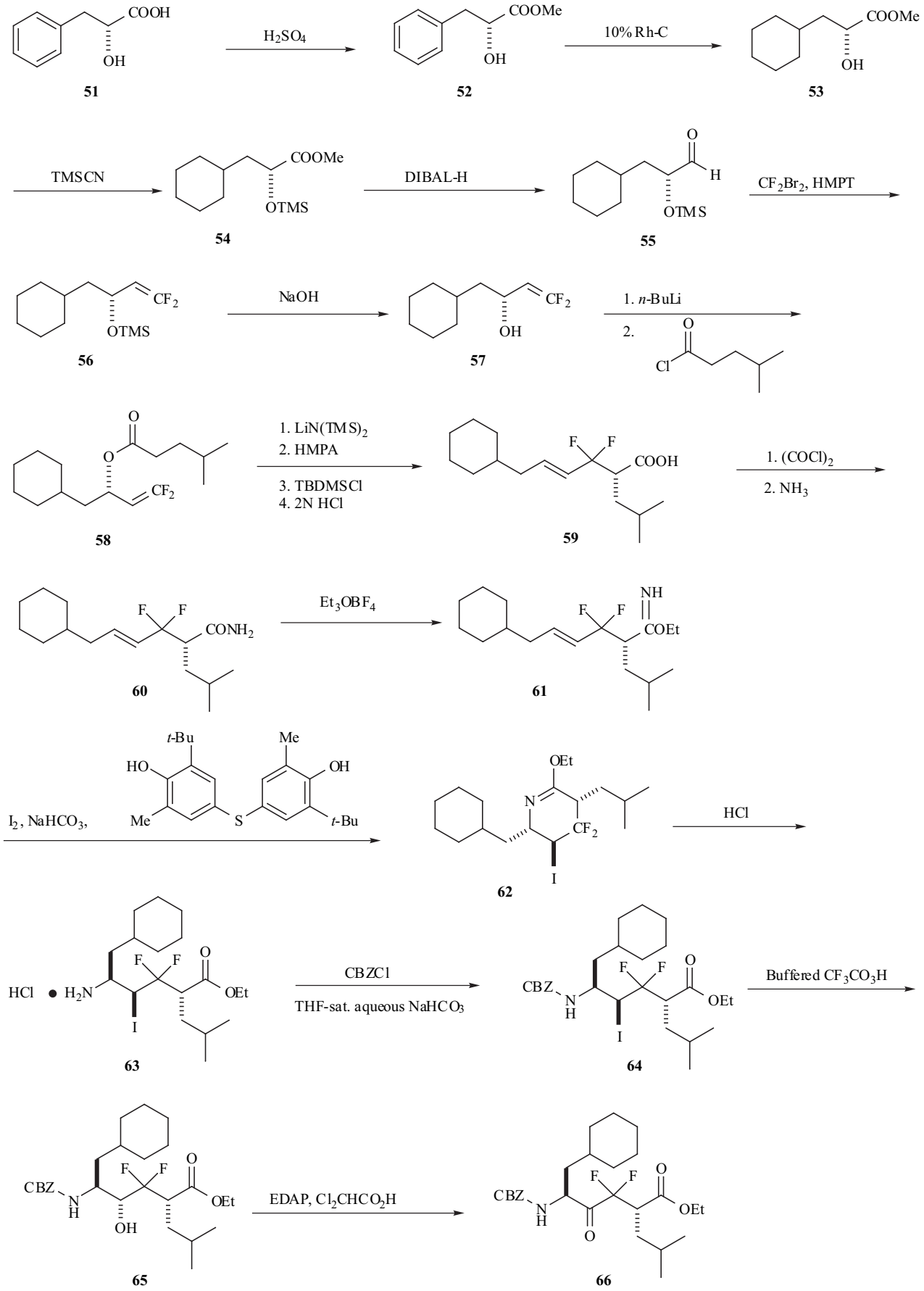


48 **49**

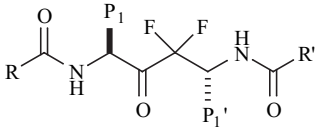


48 **50**

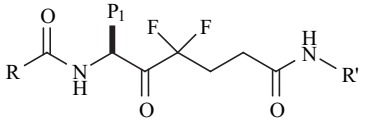
Scheme 5. [ref. 33].



Scheme 6. [ref. 35].



67



68

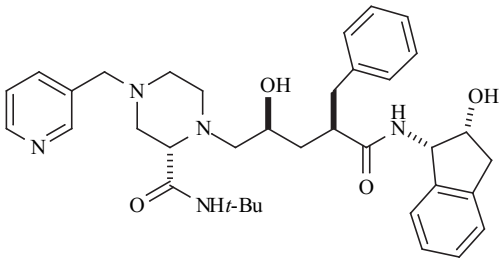
Fig. (12). Other classes of difluoroketone peptidomimetics.

HMPA, TBDMSiCl, and hydrolysis using 2 M HCl to give **59** in excellent yields. The carboxylic acid **59** was converted to the acid chloride and then to the amide **60**, which upon *O*-alkylation using triethyloxonium tetrafluoroborate produced the imino ester **61** in 95% yield. The reaction of **61** with iodine, $NaHCO_3$ and 4,4'-thio-*bis*(2-*tert*-butyl-6-methylphenol) gave a diastereomeric mixture (11:1) of the cyclic imidate **62** in 83% yield. The hydrolysis of **62** to the hydrochloride salt **63** followed by CBz protection of the amino group gave compound **64**. The iodide **64** was then subjected to a stereospecific hydrolysis to give the *N*-protected amino alcohol **65** in 73% yield. The alcohol was oxidized to give the target ketodifluoromethylene dipeptide isostere **66** in 78% yield [35]. This is a 15-step long procedure and involves some difficult steps thus making such difluoromethylene derivatives difficult to access and investigate enzyme modulation. An alternative procedure for the synthesis of ketodifluoromethylene dipeptide isosteres was achieved by Hong and co-workers employing ring-opening of oxazolones [34]. Other difluoro ketone mimics include the difluoromethyleneketone retroamide **67** proposed by Schirlin and co-workers and difluoromethyleneketone ethyl amide **68** (Fig. **12**) [33,36].

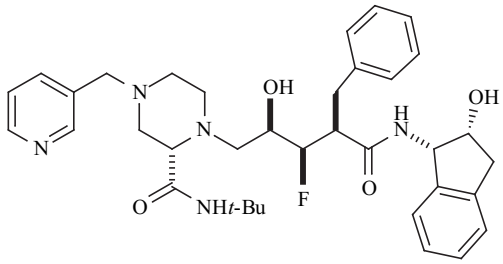
These difluorinated peptidomimetics have also been used as probes of the mechanism of protease enzymatic catalysis such as in the work performed by Wolfe and co-workers using molecular modeling and difluorostatone derivatives in biochemical analyses to classify Alzheimer's γ-secretase as an aspartyl protease [37-39].

3. THE CF MOIETY

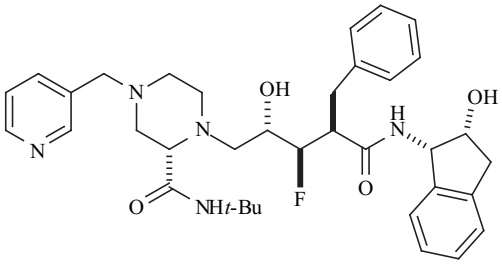
According to Myers and co-workers, in 2001, an analysis of marketed fluorine-containing drugs revealed 107



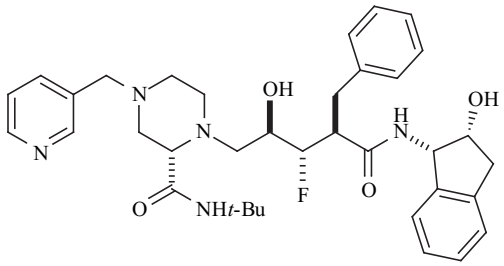
indinavir (**69**)



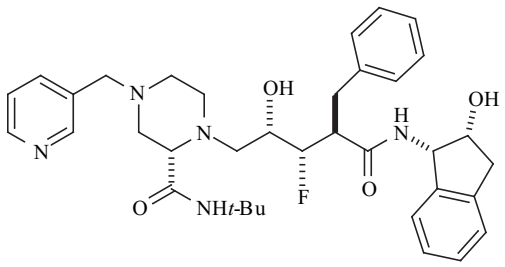
sy,syn-70



anti,syn-70



anti,anti-70

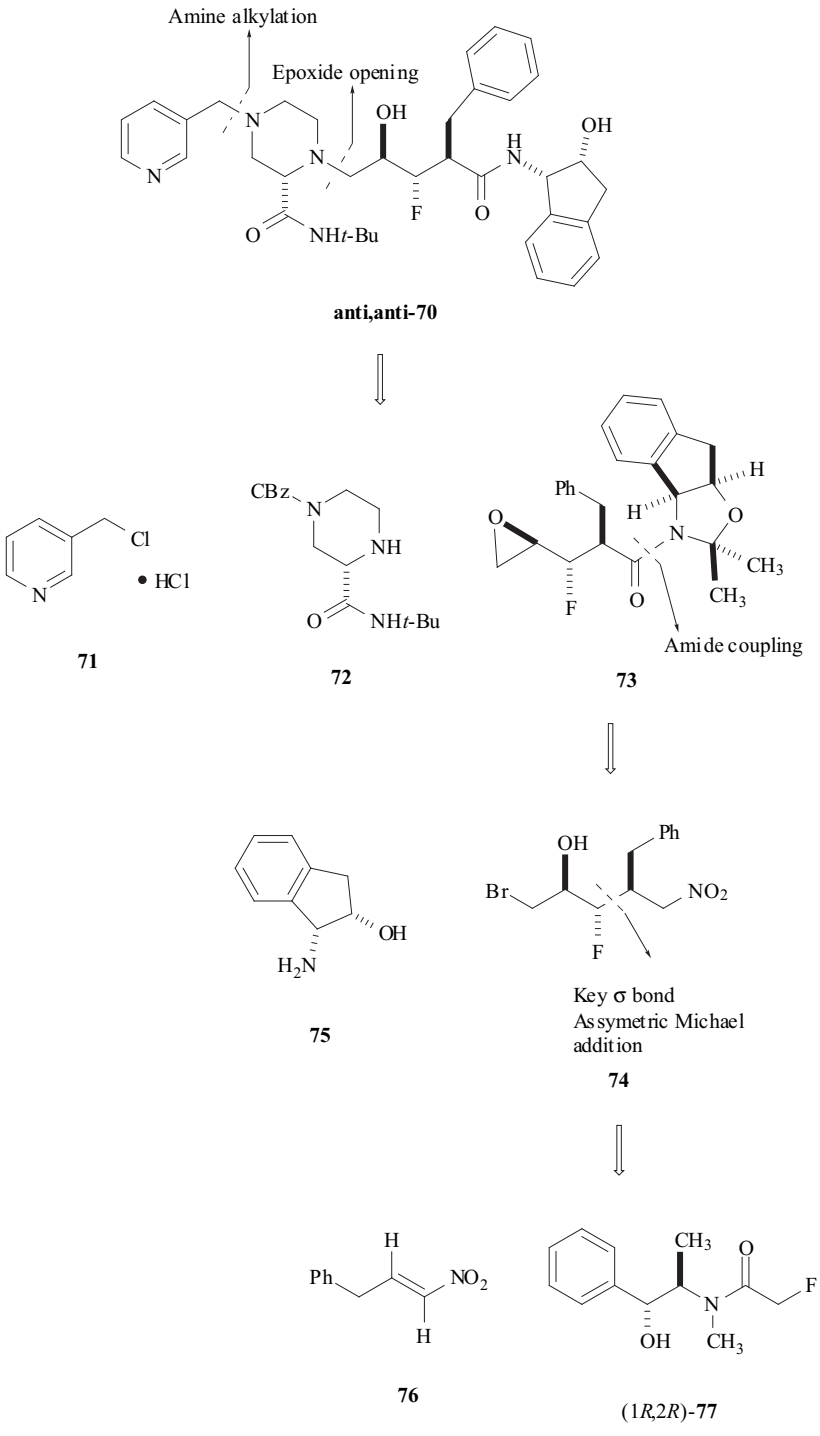


syn,anti-70

Fig. (13). Fluorinated analogs of indinavir (**69**).

compounds of which only eight contained an sp^3-hybridized carbon atom bearing a fluorine atom. The reason for this observation was attributed to the lack of viable methods for the synthesis of chiral organofluorine compounds, especially for the formation of fluorocarbon-carbon bonds [6]. Currently, one of the more common approaches employed in the introduction of fluorine into a molecule involves the use of electrophilic fluorinating agents, some of which are stable, user-friendly and commercially available [40].

HIV protease has been an attractive target for the development of new, effective antiviral agents over a number of years. The modification of a compound's properties by a fluorine atom was taken into account in the development of novel HIV protease inhibitors by Myers and co-workers [6]. Fluorine substitution as an attempt to create new, potent HIV protease inhibitors has been investigated in other studies [41,42]. Myers and co-workers designed and synthesized compounds containing a fluorinated hydroxyethylene dipeptide isosteres [6]. Dreyer and co-workers in 1989 had demonstrated that peptidomimetics containing hydroxyethylene isosteres were more potent than the analogs of statine, phosphinic acids, reduced amide

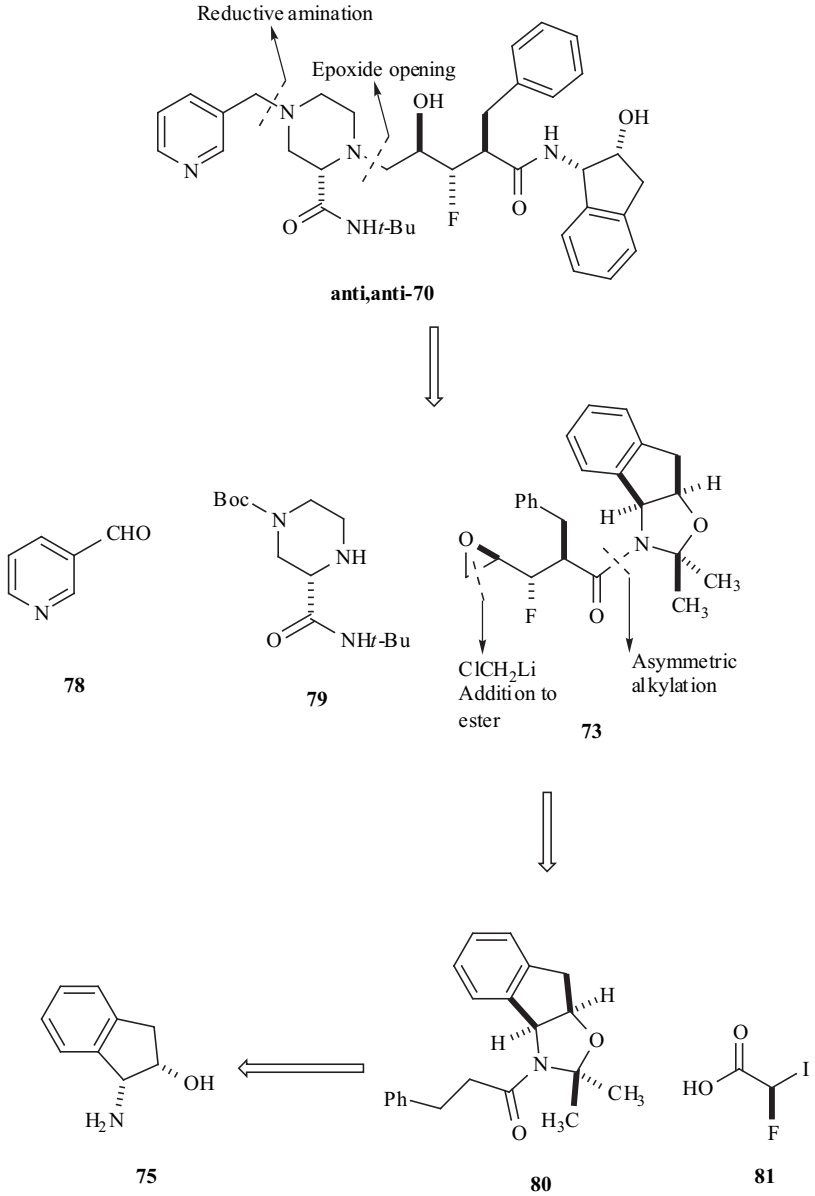


Scheme 7. [ref. 6].

isosteres and α,α-difluoroketones against the HIV-1 protease [43]. Drugs containing this isostere such as saquinavir, ritonavir, indinavir, nelfinavir and amprenavir are all inhibitors of HIV protease, and these drugs are currently used in extending the life of AIDS patients [2,6]. Fluorine substitution *alpha* to the hydroxyl group of indinavir (**69**) was achieved by Meyers and co-workers to generate the four diastereomers syn,syn-**70**, anti-syn-**70**, anti,anti-**70** and syn,anti-**70** (Fig. **13**). These diastereomers also demonstrated the effect of the stereochemistry of the fluorinated center on inhibitor-enzyme interaction (*vide infra*).

Various diastereomers of **70** were generated by employing two different methodologies. Using anti,anti-**70**, for example, the first synthetic procedure involved the C-N fragment assembly outlined in the retrosynthetic analysis of Scheme **7**. The key fragments are compounds **71**, **72**, **74** and **75**. 3-Picolyl chloride hydrochloride (**71**) is commercially available. The practical methods for the synthesis of piperazine **72** and the amino alcohol **75** are available in the literature. The synthesis of the precursor of key fragment **74** was achieved using asymmetric Michael addition of nitroalkene **76** to the lithium enolate of (1*R*,2*R*)-pseudoephedrine α-fluoroacetamide (**77**) in a moderately diastereoselective reaction. Hydrolysis of the acetamide **77** to a carboxylic acid followed by conversion of the acid to a bromide *via* a diazomethyl ketone and reduction yielded compound **74**. Syn,anti-**70** was obtained from the oxidation and reduction of the precursor to anti,anti-**70,** an acetonide-protected hydroxyamide. Syn,syn-**70** was synthesized using the enantiomer of (1*R*,2*R*)-**77,** and anti,syn-**70** was obtained in a similar manner to syn,anti-**70** [6].

The second-generation synthesis of compounds **70** was accomplished using a stereospecific alkylation of the enolate derived from aminoindanol hydrocinnamide (**80**) to an optically active fluoroiodoacetic acid (**81**) to give the key intermediate **73** (Scheme **8**). Biological activity assays of the four diastereomers **70** and indinavir (**69**) against HIV-1 protease revealed syn,syn-**70** to be the most potent of the

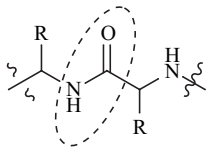


Scheme 8. [ref. 6].

fluorinated inhibitors. At a K_i of 2 nM, it was equipotent to indinavir (**69**) with a K_i of 1.9 nM [6]. The other diastereomers, which were more lipophilic compared to indinavir and syn,syn-**70**, were less potent with inhibition constants varying over a range of 3 orders of magnitude. While these results illustrated the property-modifying effects of fluorine substitution, the importance of stereochemistry on inhibitor-enzyme binding was also reinforced in these studies [6].

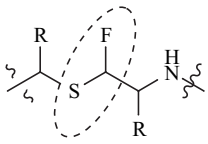
Our laboratory has been interested in devising novel approaches to inhibit proteases. The term "fluoropeptidomimetics" was used to describe a novel approach to protease inhibition where the scissile peptide bond '-CO-NH-' of the native peptide substrate **82** is mimicked by a '-CHF-S-' moiety [44]. We proposed that the key peptidomimetic moiety **83** resembles the tetrahedral transition-state oxyanion species **84** formed during the hydrolysis of a peptide bond by a serine protease, thus inhibiting the enzyme activity (Scheme **9**). These peptidomimetics could inhibit the protease *via* one of the following modes: as transition-state mimics, slow, tight-binding inhibitors, or covalent irreversible inhibitors *via* the formation of the sulfenium species **86** (Scheme **9**, II). It is anticipated that the fluorine is stabilized in the active site of serine proteases through hydrogen bonding, similar to an oxyanion in the catalytic site of a protease. This interaction may result in the elimination of fluorine facilitated by the lone pair of electrons on sulfur, generating the reactive species **86** that is predisposed for a nucleophilic attack by an active site residue (Scheme **9**) [44].

Our first synthetic approach en route to the target compounds centered on α-fluoroglycine derivatives such as compound **88** (Scheme **10**). We envisioned that we could arrive at the target amino intermediate **87** either from precursors such as compound **88** or α-fluoro-β-aminoethanethiol derivatives (**89**). In the context of peptidomimetics, the α-fluorination of amino acids has been of interest to several research groups, however the unstable nature of the '-NH-CHF-' moiety have made the α-fluoro-α-amino acids difficult to isolate [45]. Rather, only α-fluoroglycine derivatives containing functionalized amines such as an azido group, a nitro group, a tertiary or quaternary amine, which prevent decomposition through spontaneous dehydrofluorination, have been synthesized [45,46]. We reported the synthesis and isolation of α-fluoro-*N*-benzyl glycine ethyl ester (**92**) for the first time. While compound **92** was successfully synthesized from the treatment of benzylamine (**90**) with ethyl bromofluoroacetate (**91**) in anhydrous DMF (Scheme **11**), further attempts to derivatize **92** en route to the amino derivative **87** were unsuccessful



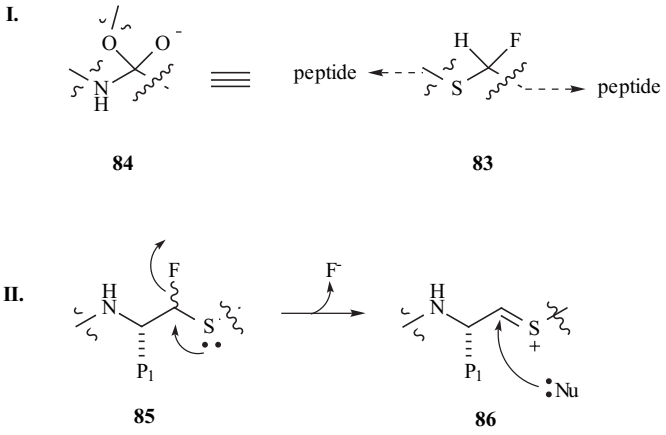
native peptide substrate, R = side chain

82



fluoropeptidomimetic analogue, R = side chain

83

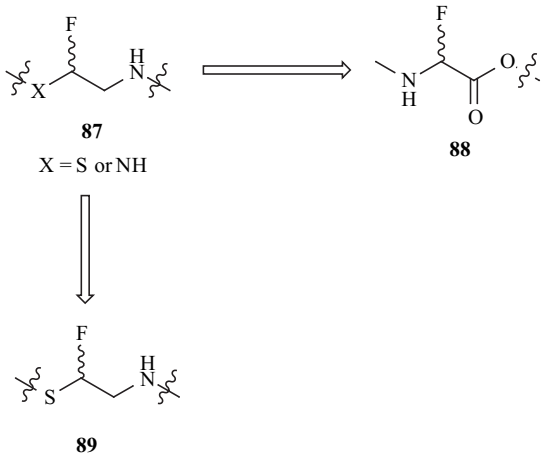


84 **83**

85 **86**

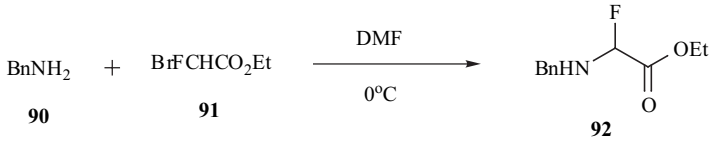
Scheme 9. [ref. 45,48].

[44]. Hence we centered our attention on α-fluoro-β-aminoethanethiol derivatives such as **89**. One successful synthetic strategy involved electrophilic fluorination at the *alpha* position to the sulfide group using the commercially available Selectfluor^{TM} as shown in Scheme **12** [44,47]. Reduction of the amino acid to the amino alcohol of **93** followed by *N*-phthaloyl protection yielded compound **94**. This was followed by thiolate substitution of the alcohol moiety to give compound **95**, which was fluorinated *alpha* to the sulfide. The resulting fluoro monomer **96** was deprotected to yield free amine **97** for peptide coupling. Phthalimide was chosen as the most suitable protecting group primarily due to the labile nature of the fluoropeptidomimetics towards acidic conditions, and the suitability of the mild conditions used in its deprotection.

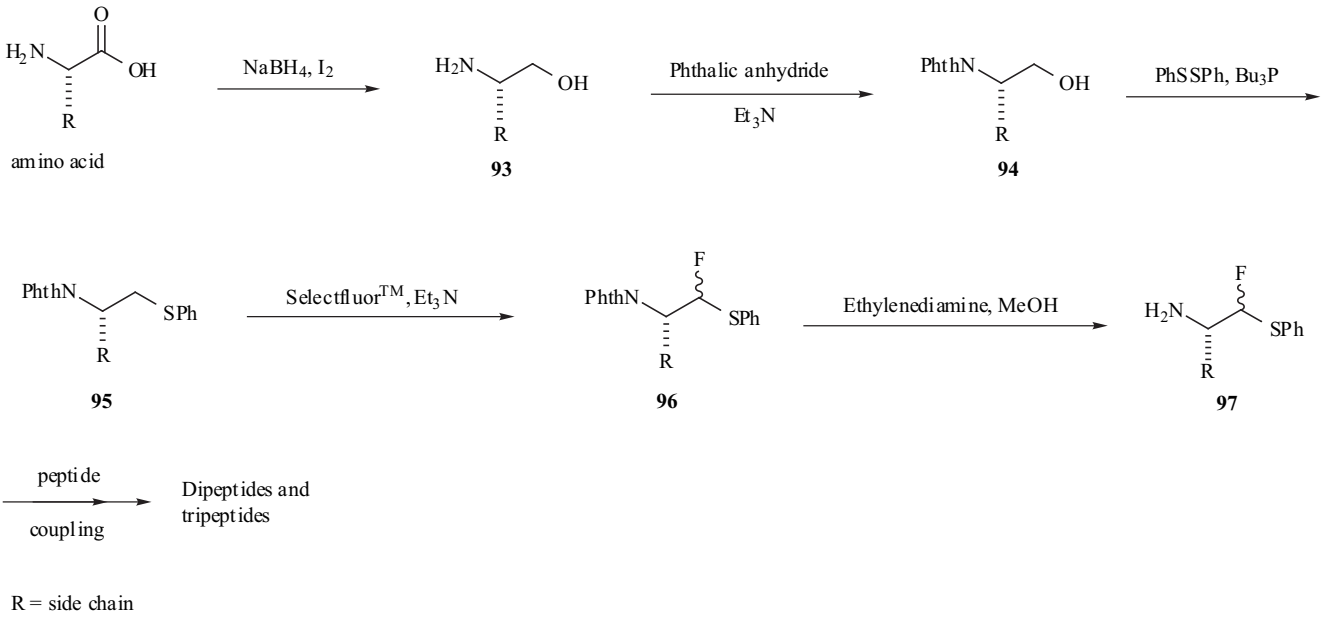


Scheme 10. [ref. 45].

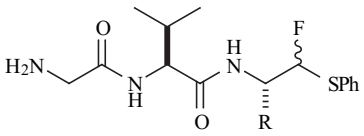
A comprehensive set of dipeptide and tripeptide derivatives were synthesized, their stability in aqueous media was determined. The activity of tripeptides **98-102** against chymotrypsin was evaluated (Fig. **14**). Computational modeling showed that, like the tetrahedral oxyanion, in an energy-minimized complex of compound **99** and chymotrypsin, the fluorine atom is hydrogen bonded to the backbone nitrogen of Gly 193 of the oxyanion hole at a distance of 2.98 Å, and is in close proximity to the catalytic serine residue. Based on quantitative stability studies performed using ^{19}F NMR, several conclusions were drawn: (i) alkyl substitutions *alpha* to fluorine enhanced the stability of the compounds in aqueous media possibly through steric and/or electronic effects; (ii) electron donating substituents on the phenyl ring reduced stability; and (iii) increasing peptide length improved the aqueous stability of the compounds. Qualitative stability studies were carried out on tripeptides **98**, **99** and **101** by stirring in 50% aqueous methanol. After 4 days, 5-substituted-2-thiophenyloxazoles **104** were obtained as the major decomposition products, leading to the synthesis of deoxy tripeptide mimic **103** *via* an imine in an attempt to prevent oxazole formation [47]. However, compound **103** was found to decompose more rapidly than the tripeptides **98-102** yielding the methyl ether **105** (Fig. **15**). Biological assays of the tripeptides against chymotrypsin revealed that compounds **98** and **99**, both containing hydrophobic groups at P1, showed time-dependent loss of activity with a maximum inhibition of 67% and 79%, respectively, and dissociation constants (K_i) of 63 and 120 μM, respectively. These results are the first in the synthesis of rationally designed peptidomimetics substituting the traditional carbonyl-like groups with fluorine for transition-state mimicry.



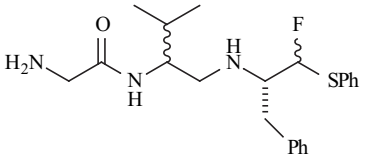
Scheme 11. [ref 45].



Scheme 12. [ref. 48].

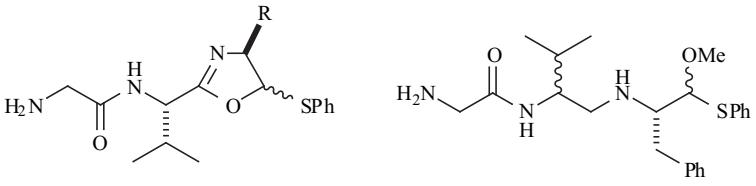


98, R = CH_2Ph
99, R = CH_2(cyclohexyl)
100, R = $CH(CH_3)_2$
101, R = $CH_2CH(CH_3)_2$
102, R = $CH(CH_3)C_2H_5$



103

Fig. (14). Tripeptide mimetics **98 - 103**.



104 **105**

R = CH_2Ph
CH_2(cyclohexyl)
$CH_2CH(CH_3)_2$

Fig. (15). Aqueous decomposition products of the tripeptide mimics after 4 days in 50% aqueous methanol.

4. CONCLUSION

Above mentioned approaches in the context of designing novel peptidomimetics with fluorines as well as designing novel fluorinated protease inhibitors are important steps in organic synthesis adopting elegant chemical synthetic methods to understand enzymes. Useful strategies of such kind have lead and could lead to potential biologically active compounds as drugs modulating a disease condition. The use of fluorine to modulate bioactivity is only going to be more frequent as the organic chemistry community discovers new means of incorporating fluorine atoms easily into various templates of peptide-like structures.

ACKNOWLEDGEMENTS

Authors acknowledge the generous support from the Canadian Institutes of Health Research (LPK). LPK is a recipient of Rx&D HRF-CIHR research career award and Premier's Research Excellence Award (PREA). CEO is partially supported by the PREA.

REFERENCES

[1] Saitton, S.; Kihlberg, J.; Luthman, K. *Tetrahedron* **2004**, *60*, 6113.
[2] Ripka, A.; Rich, D. *Cur. Opin. Chem. Biol.* **1998**, *2*, 441.
[3] Leung, D.; Abbenante, G.; Fairlie, D. *J. Med. Chem.* **2000**, *43*, 305.
[4] Carte, D.; Chae, C-B. *Biochemistry* **1976**, *15*, 180.
[5] (a) Marais, J. S. C. *Onderstepoort J. Vet. Res.* **1943**, *18*, 203. (b) Marais, J. S. C. *Onderstepoort J. Vet. Res.* **1944**, *20*, 67-73.
[6] Myers, A.; Barbay, J.; Zhong, B. *J. Am. Chem. Soc.* **2001**, *123*, 7207, and references therein.
[7] Ismail, F. *J. Fluor. Chem.* **2002**, *118*, 27.
[8] Fried, J.; Sabo, E. *J. Am. Chem. Soc*. **1954**, *76*, 1455.
[9] Smart, B. *J. Fluor. Chem.* **2001**, *109*, 3.
[10] (a) Smart, B. In *Chemistry of Organic Fluor. Compounds II: A Critical Review*; Hudlicky, M.; Pavlath, A. Eds.; ACS Monograph 187; American Chemical Society: Washington DC, **1995**; pp. 979-1010. (b) Smart, B. In *OrganoFluor. Chemistry: Principles and Commercial Applications*; Banks, R.; Smart, B.; Tatlow, J. Eds.; Plenum Publishing Corporation: New York, **1994**; pp. 57-88.
[11] (a) Ohba, T.; Ikeda, E.; Takei, H. *Bioorg. Med. Chem*. **1996**, *6*, 1875. (b) Kashima, A.; Inove, Y.; Sugio, S.; Maeda, I.; Nose, T.; Shimoshigashi, Y. *Eur. J. Biochem*. **1998**, *255*, 12. (c) Parisi, M.; Abeles, R. *Biochemistry* **1992**, *31*, 9429.
[12] Zanda, M. *New J. Chem.* **2004**, *28*, 1401.
[13] Mikami, K.; Itoth, Y.; Yamanaka, M. *Chem. Rev.* **2004**, *104*, 1.
[14] Park, B.; Kitteringham, N.; O'Niel, P. *Annu. Rev. Pharmacol. Toxicol.* **2001**, *41*, 443.
[15] Moletti, M.; Pesenti, C.; Sani, M.; Volonterio, A.; Zanda, M. *J. Fluor. Chem.* **2004,** *125*, 1735.
[16] Ismail, F.; Dascombe, M.; Carr, P.; North, S. *J. Pharm. Pharmacol.* **1996,** *48*, 841.
[17] Coussens, L.; Fingelton, B.; Matrisian, L. *Science* **2002,** *295*, 2387. (b) Whittaker, M.; Floyd, C.; Brown, P.; Gearing, A. *Chem Rev.* **1999,** *99*, 2735. (c) Giavazzi, R.; Taraboletti, G. *Cri. Rev. Oncol. Hematol.* **2001,** *37*, 53.
[18] Babine, R.; Bender, S. *Chem. Rev.* **1997,** *97*, 1359.
[19] Volonterio, A.; Bellosta, S.; Bravo, P.; Canavesi, M.; Corradi, E.; Meille, S.; Monetti, M.; Moussier, N.; Zanda, M. *Eur. J. Org. Chem.* **2002**, 428.

[20] Volonterio, A.; Bellosta, S.; Bravin, F.; Belucci, M.; Bruche, L.; Colombo, G.; Malpezzi, L.; Mazzini, S.; Meille, S.; Meli, M.; Ramirez de Arellano, C.; Zanda, M. *Chem. Eur. J.* **2003**, *9*, 4510.
[21] Jacobson, I.; Reddy, G. *Tetrahedron Lett.* **1996**, *37*, 8263.
[22] Jacobson, I.; Reddy, P.; Wasserman, Z.; Hardman, K.; Covington, M.; Arner, E.; Copeland, R.; Decicco, C.; Magolda, R. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 837.
[23] Sani, M.; Belotti, D.; Giavazzi, R.; Panzeri, W.; Volonterio, A.; Zanda, M. *Tetrahedron Lett.* **2004,** *45*, 1611.
[24] Bell, I. *J. Med. Chem*. **2004**, *47*, 1869.
[25] (a) Augeri, D.; O'Connor, S.; Janowick, D.; Szczepankiewicz, B.; Sullivan, G.; Larsen, J.; Kalvin, D.; Cohen, J.; Devine, E.; Zhang, H.; Cherian, S.; Saeed, B.; Ng, S.-C; Rosenberg, S. *J. Med. Chem,* **1998,** *41*, 4288. (b) O'Connor, S.; Barr, K.; Wang, L.; Sorensen, B.; Tasker, A.; Sham, H.; Ng, S.-C.; Cohen, J.; Devine, E.; Cherian, S.; Saeed, B.; Zhang, H.; Lee, J.; Warner, R.; Tahir, S.; Kovar, P.; Ewing, P.; Alder, J.; Mitten, M.; Leal, J.; Marsh, K.; Bauch, J.; Hoffman, D.; Sebti, S. *J. Med. Chem*. **1999**, *42*, 3701. (c) Henry, K., Jr.; Wasical, J.; Tasker, A.; Cohen, J.; Ewing, P.; Mitten, M.; Larsen, J.; Kalvin, D.; Swenson, R.; Ng, S.-C.; Saeed, B.; Cherian, S.; Sham, H.; Rosenberg, S. *J. Med. Chem*. **1999,** *42*, 4844.
[26] Wang, G.; Wang, X.; Wang, W.; Hasvold, L.; Sullivan, G.; Hutchins, C.; O'Conner, S.; Gentiles, R.; Sowin, T.; Cohen, J.; Gu, W.-Z.; Zhang, H.; Rosenberg, S.; Sham, H. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 153.
[27] Kuznetsova, L.; Ungureanu, I.; Pepe, A.; Zanardi, I.; Wu, X.; Ojima, I. *J. Fluor. Chem.* **2004,** *125*, 487.
[28] Thaisrivongs, S.; Pals, D.; Kati, W.; Turner, S.; Thomasco, L.; Watt, W. *J. Med. Chem.* **1986**, *29*, 2080.
[29] Marciniszyn, J.; Hartsuck, J.; Tang, J. *J. Biol. Chem*. **1976**, *251*, 7088.
[30] Rich, D.; Bopari, A.; Bernatowicz, M. *Biochem. Biophys. Res. Commun*. **1982**, *104*, 1127.
[31] Eda, M.; Ashimori, A.; Akahoshi, F.; Yoshimura, T.; Inoue, Y.; Fukaya, C.; Nakajima, M.; Fukuyama, H.; Imada, T.; Nakamura, N. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 913.
[32] Kinoshita, A.; Urata, H.; Bumpus, F.; Husain, A. *J. Biol. Chem.* **1991**, *266*, 19192.
[33] Schirlin, D.; Baltzer, S.; Altenburger, J.; Tarnus, C.; Remy, J. *Tetrahedron* **1996**, *52*, 305.
[34] Hong, W.; Dong, L.; Cai, Z.; Titmas, R. *Tetrahedron Lett.* **1992**, *33*, 741.
[35] Damon, D.; Hoover, D. *J. Am. Chem. Soc*. **1990**, *112*, 6439 and references therein.
[36] Imperiali, B.; Abeles, R. *Biochemistry* **1987**, *26*, 4474.
[37] Wolfe, M.; Citron, M.; Diehl, T.; Xia, W.; Donkor, I.; Selkoe, D. *J. Med. Chem*. **1998**, *41*, 6.
[38] Wolfe, M.; Xia, W.; Moore, C.; Leatherwood, D.; Ostaszewski, B.; Rahmati, T.; Donkor, I.; Selkoe, D. *Biochemistry* **1999**, *38*, 4720.
[39] Moore, C.; Leatherwood, D.; Diehl, T.; Selkoe, D.; Wolfe, M. *J. Med. Chem*. **2000**, *43*, 3434.
[40] For a review of electrophilic fluorination, see (a) Taylor, S.; Kotoris, C.; Hum, G. *Tetrahedron* **1999**, *55*, 12431. (b) Banks, R. *J. Fluor. Chem.* **1998**, *87*, 1. (c) Lal, G. S.; Pez, G.; Syvret, R. *Chem. Rev*. **1996**, *96*, 1737.
[41] Lindberg, J.; Pyring, D.; Löwgren, S.; Rosenquist, Å.; Zuccarello, G.; Kvarnström, I.; Zhang, H.; Vrang, L.; Classon, B.; Hallberg, A.; Samuelsson, B.; Unge, T. *Eur. J. Biochem.* **2004,** *271*, 4594.
[42] Pyring, D.; Lindberg, J.; Rosenquist, Å.; Zuccarello, G.; Kvarnström, Zhang H.; Vrang, L.; Unge, T.; Classon, B.; Hallberg, A.; Samuelsson, B. *J. Med. Chem*. **2001,** *44*, 3083.
[43] Dreyer, G.; Metcalf, B.; Tomaszker Jr., T.; Carr, T.; Chandler III, A.; Hyland, L.; Fakhoury, S.; Magaard, V.; Moore, M.; Strickler, J.; Debouck, C.; Meek, T. *Proc. Natl. Acad. Sci. USA* **1989**, *86*, 9752.
[44] Annedi, S.; Li, W.; Samson, S.; Kotra, L. *J. Org. Chem*. **2003**, *68*, 1043.
[45] (a) Takeuchi, Y.; Takagi, K.; Nagata, K.; Koizumi, T. *Chem.. Pharm. Bull*. **1991**, *39*, 3120. (b) Takeuchi, Y.; Takagi, K.; Yamaba, T.; Nabetani, M.; Koizumi, T. *J. Fluor. Chem.* **1994**, *68*, 149.
[46] (a) Bailey, P.; Baker, S.; Boa, A.; Clayson, J.; Rosair, G. *Tetrahedron Lett*. **1998**, *39*, 7755. (b) Bailey, P.; Boa, A.; Crofts, G.; Van Diepen, M.; Helliwell, M.; Gammon, R.; Harrison, M. *Tetrahedron Lett*. **1989**, *30*, 7457. (c) Takeuchi, Y.; Nabetani, M.; Takagi, K.; Hagi, T.; Koizumi, T. *J. Chem. Soc*., *Perkin Trans. 1* **1991**, 49.
[47] Annedi, S.; Majumder, K.; Wei, L.; Oyiliagu, C.; Samson, S.; Kotra, L. *Bioorg. Med. Chem*. **2005**, *13*, 2943.

Received: September 19, 2005 **Revised: October 22, 2005** **Accepted: October 29, 2005**