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Review

Solution and solid-phase synthesis of trifluoromethyl peptides and mimetics

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Abstract

This paper reviews the solution-phase synthesis of trifluoromethyl (Tfm)-analogues of bioactive peptides, such as RGD-peptides and the aspartyl protease inhibitor pepstatin, and the solution/solid-phase synthesis of Tfm-substituted retro- and retro-inverso peptides and hydroxamates. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Trifluoromethyl group; Asymmetric synthesis; Solid-phase synthesis; Peptides; Peptidomimetics

1. Introduction

Since 1997, as a natural evolution of previous work on the asymmetric synthesis of fluorinated amino acids (AAs) [1], we started a research program directed toward the solution/solid-phase synthesis of fluorinated peptides and mimetics, with an emphasis on the development of synthetic methods suitable for combinatorial applications, and the investigation of the effect of fluoro-substitution on the activity of target enzymes. This paper reviews our results concerning (1) the solution-phase synthesis of analogues of bioactive peptides, such as RGD and pepstatin, incorporating α-trifluoromethyl (Tfm)-α-amino- and γ-Tfm-γ-amino acids, (2) the solution/solid-phase synthesis of Tfm-substituted retro- and retro-inverso peptides, including retro-peptidyl hydroxamates.

Abbreviations: GABOB, γ -amino-β-hydroxybutyric acid; EDCI, *N*-ethyl-*N′*-(3-dimethyl-aminopropyl)carbodiimide hydrochloride; HOBt, 1-hydroxybenzotriazole; HATU, *N,N,N′,N′*-tetramethyl-*O*-(7-azabenzotriazol-1-yl)uronium hexafluorophosphate; HOAt, 1-hydroxy-7-azabenzotriazole; TMP, 2,4,6-trimethylpyridine (*sym*-collidine); NMM, *N*-methylmorpholine; Boc, *tert*-butoxycarbonyl; Cbz, carbobenzyloxy; Bn, benzyl; TIB, 1,1-bis-(trifluoroacetoxy)-iodobenzene; TFA, trifluoroacetic acid; DCC, dicyclohexylcarbodiimide; RP, reverse phase; AA, amino acid; DIC, diisopropylcarbodiimide

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2. Synthesis of peptides incorporating trifluoromethyl-amino acids

2.1. RGD analogues incorporating α-trifuoromethyl-α-amino acids

Rapid degradation, low lipophilicity, low permeability through cell membranes, low selectivity due to high conformational flexibility are only some of the drawbacks of peptide drugs. A possible improvement could arise from incorporation of α-Tfm-α-amino acids into key positions of peptides [2]. In fact, the high electronegativity, high lipophilicity, locally hydrophobic character, and high steric demand of the Tfm group have been shown to bring about specific modifications of some properties, such as retarded degradation by peptidases, in vivo absorption through certain body barriers, and severe conformational restrictions inducing well-defined secondary structures. Moreover, the Tfm group, owing to its high electron-density, may act as a coordinative site with the receptor, or offer a further chance to the substrate to engage in hydrogen bonding. Last but not least, the possibility to investigate metabolic processes and conformational properties of Tfm-containing proteins and peptides via ^{19}F NMR represents a powerful analytic tool.

An extremely important biological target is represented by the sequence Arg–Gly–Asp (RGD) [3], which represents a contributing factor in the platelet-mediated thrombus formation. Enormous interest has been devoted to the discovery of RGD analogues for an anti-thrombotic therapy [4].

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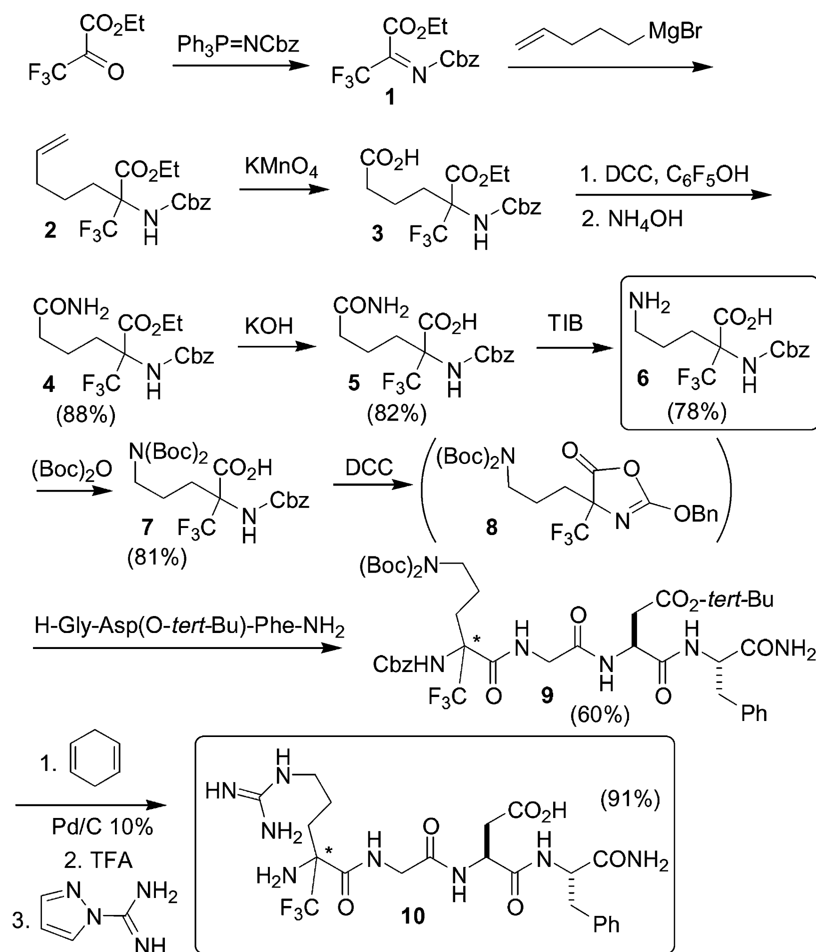


Scheme 1.

In connection with a program for the synthesis of new RGD-peptide mimetics incorporating α-Tfm-α-AAs [5], as conformational modifiers, we have studied in collaboration with the group of A.Dal Pozzo, the synthesis of RGD-peptides incorporating α-Tfm-α-AAs. The synthesis of an RGD analogue incorporating a *N*-terminal Tfm-arginine is portrayed in Scheme 1 [6]. The racemic precursor Tfm-ornithine (**6**) was synthesized by a multi-step process starting from the *N*-Cbz (carbobenzyloxy) imine of trifluoropyruvate (**1**), which underwent addition of *n*-pent-4-enylmagnesium bromide affording **2**. Oxidative demolition by means of $KMnO_4$ to the acid **3**, followed by transformation into the amide **4**, alkaline hydrolysis of the ester to the acid **5**, and Hoffman degradation gave the N_α-protected Tfm-ornithine (**6**), which was protected as *tert*-butoxycarbonyl $(Boc)_2N_\omega$ derivative **7**. The carboxylic group of **7** was activated via transformation into the intermediate oxazol-5-one (**8**) and submitted to coupling with H-Gly-Asp(*O-tert*-Bu)-Phe-NH_2, producing the protected Tfm-Orn-containing tetrapeptide (**9**). Deprotections and guanylation afforded the Tfm-arginine-containing RGD analogue **10**.

Using a similar strategy, a further RGD-peptide having a terminal phenetylamide residue (**11**) was prepared (Fig. 1). In both cases **10** and **11** diastereomerically pure samples were purified by RP-HPLC.

We were also able to prepare RGD-peptides incorporating a Tfm-aspartic acid at the P^3 position (Scheme 2) [7]. This result is remarkable because incorporation of Tfm-AAs into peptides through their amino group is a challenging endeavour, due to its poor nucleophilicity. Addition of allylmagnesium chloride to the imine **1** (see Scheme 1) provided **12**, that was submitted to alkaline hydrolysis to the acid **13**, which was coupled with H-Phe-NH_2 through an intermediate oxazol-5-one, delivering the dipeptide **14**. After cleavage of the Cbz group, the critical coupling was achieved in high yields upon treatment of **15** with *N*-phthaloylglycine chloride. The allyl side chain of the resulting tripeptide **16** was

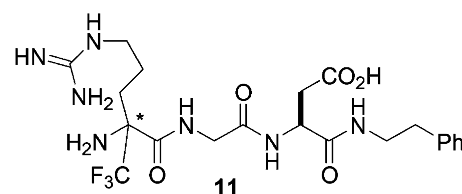
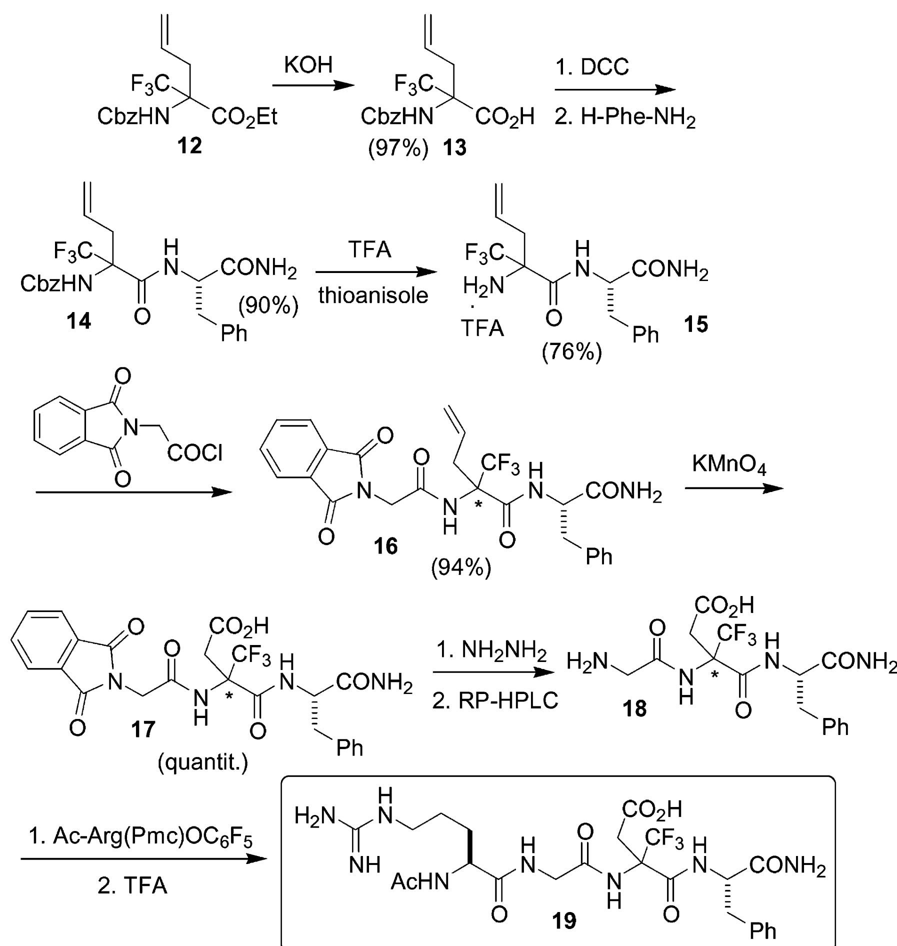


Fig. 1.



Scheme 2.

submitted to oxidative demolition with $KMnO_4$, affording the peptide **17** incorporating Tfm-Asp. After removal of the phtalimide group with hydrazine, pure diastereomers of the NH_2-free peptide **18** were obtained via RP-HPLC, and separately submitted to coupling with a suitably protected arginine. The resulting diastereomerically pure protected peptides were finally treated with trifluroracetic acid (TFA), affording the target Tfm-Asp RGD analogues **19**. Biological data on the activity of Tfm-RGD-peptides **10**, **11** and **19** are unfortunately unavailable.

2.2. Pepstatin analogue incorporating a γ-trifluoromethyl-γ-amino acid

Pepstatin (Iva–Val–Val–Sta–Ala–Sta, Fig. 2) is a naturally occurring inhibitor for aspartic proteases which contains two units of the unusual AA (4S,3S)-statine [8].

Its binding is often characterized by dissociation constants in the range of 0.1–1 nM [9–11], with the exceptions of renin [12,13] and HIV-1 protease [14–20], for which pepstatin is less inhibitory ($IC_{50} = 0.32$ and 2.5 μM, respectively). Kinetic studies have shown that the (3S)-hydroxyl group of the central statine is important for tight binding of

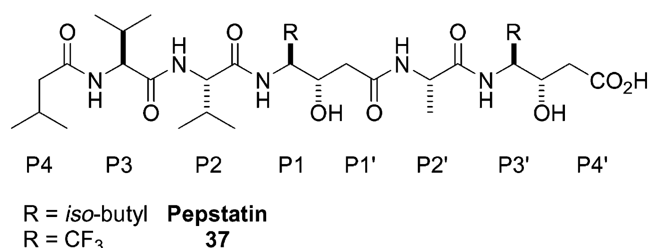
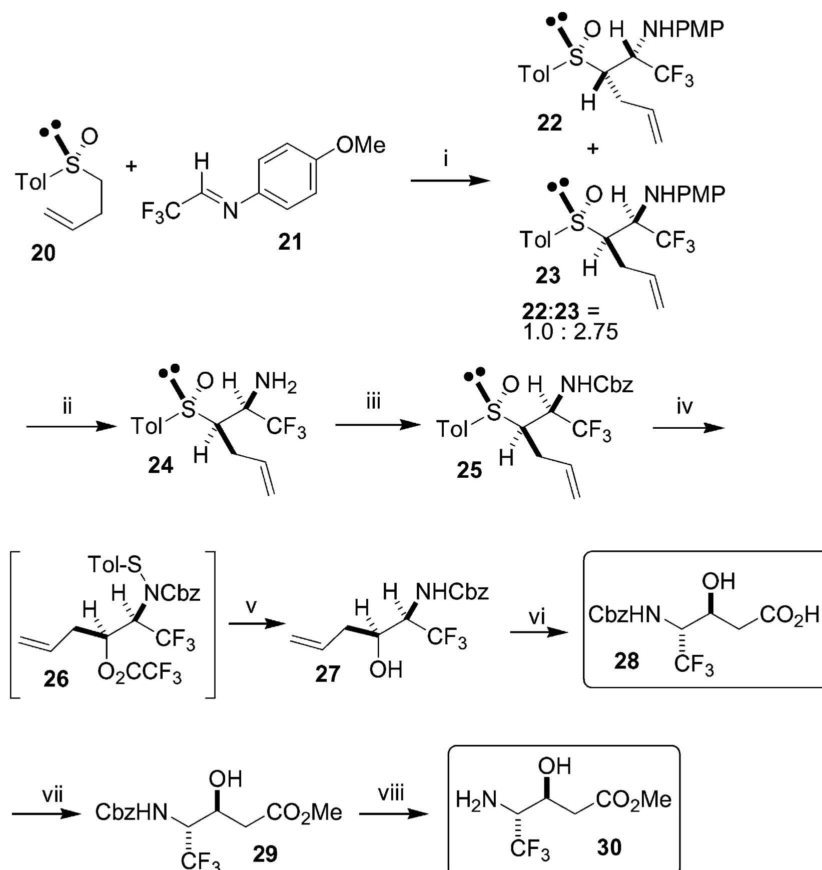


Fig. 2.

pepstatin [21,22]. Replacement of the statine isobutyl residue in the P1 position with other groups is a strategy that has sometimes led to analogues with improved properties [10,23,24].[2] However, its replacement with a fluoroalkyl residue has never been reported, despite the fact that fluoroalkyl groups are known to deeply modify physical–chemical properties, to enhance pharmacological activity, and that useful spectroscopic data on the binding process might be obtained by ^{19}F NMR [25]. With this in mind, we have

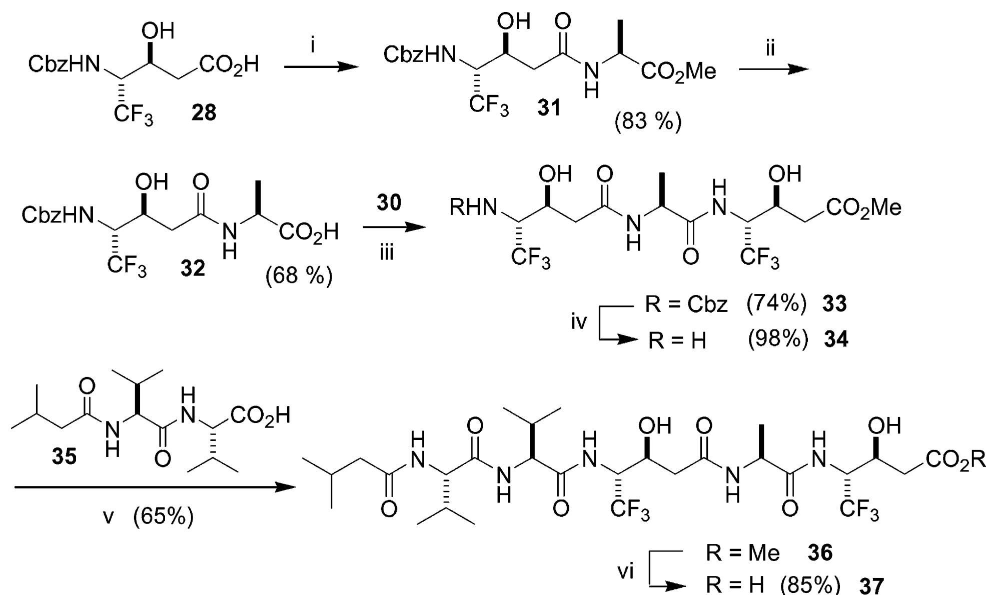
[2] Pepstatin is rated only as moderately active by NCI in a cell-based AIDS anti-viral screen.



Scheme 3. Key: (i) LDA, THF, −70°C; (ii) CAN, CH_3CN, H_2O, then FC (66%); (iii) $ClCO_2CH_2Ph$, K_2CO_3 50%, dioxane (>98%); (iv) $(CF_3CO)_2O$, *sym*-collidine, CH_3CN; (v) K_2CO_3/H_2O up to pH 7, then $NaBH_4$, THF/H_2O, 0°C (94%); (vi) $KMnO_4$, H_2SO_4 3 N, acetone/H_2O, 0°C, 15 min (70%); (vii) CH_2N_2, MeOH; (viii) $Pd(OH)_2$/C, H_2, MeOH (90%).

recently accomplished the total solution-phase synthesis of the pepstatin analogue **37** (Fig. 2), having two γ-Tfm-γ-amino-β-hydroxybutyric (GABOB) units [26] in place of the natural *syn*-(3S,4S)-statines in the P^1 and $P^{3\prime}$ positions [27,28]. A multigram stereocontrolled preparation of orthogonally protected enantio- and diasteromerically pure (3S,4R)-Tfm-analogues of statine, namely the γ-Tfm-GABOBs **28** and **30**, is described in Scheme 3. It is worth noting that exploiting a protocol based on the ‘‘non-oxidative’’ Pummerer reaction (NOPR), first developed in our laboratories, lithiated (*R*)-*p*-tolyl γ-butenyl sulfoxide (**20**) was used as a chiral 3-hydroxy-propionate 3-carbanion equivalent with the *N*-*p*-methoxyphenyl (*N*-PMP) imine of fluoral (**21**), to achieve the synthesis of the targeted *syn* (Tfm)GABOB unit [29,30]. The reaction afforded two diastereomeric *N*-PMP β-amino sulfoxides (**22**) and (**23**) out of four possible, in 1.0/2.75 d.r. and nearly quantitative overall isolated yields. The mixture of **22** and **23** was treated with ceric ammonium nitrate (CAN) to cleave the *N*-PMP group, providing the free amino sulfoxide **24** in diastereomerically pure form after flash chromatography (FC). Compound **24** was reprotected as *N*-Cbz derivative **25**, then submitted to the NOPR protocol. As expected, treatment of **25** with trifluoroacetic anhydride and *sym*-collidine (TMP) triggered a S_N2 displacement of the sulfinyl by a trifluoroacetoxy group, leading to the intermediate sulfenamide **26**. One-pot treatment with K_2CO_3 followed by $NaBH_4$, provided the β-amino alcohol **27**, with overall stereoselectivity >98/2. Oxidative cleavage with $KMnO_4$ occurred with excellent chemoselectivity providing the *N*-Cbz acid **28**. The latter was treated with diazomethane, then the Cbz group of the resulting ester **29** was hydrogenolyzed providing NH_2-free ester **30**.

As a strategy to achieve the assembling of Tfm-statine **37** (Scheme 4), the coupling of the tripeptide fragment H-(Tfm)GABOB-Ala-(Tfm)GABOB with Iva–Val–Val–OH was planned. Coupling of **28** with H–Ala–OMe afforded **31**, which was hydrolyzed to the corresponding acid **32**. Satisfactorily, the *N*-coupling of **30** to **32** occurred efficiently affording good yields of **33**, which was hydrogenolyzed to the H_2N-tripeptide **34**. It is worth noting that the coupling step leading to **32** is a critical one. In fact, a number of different strategies to achieve formation of this peptide bond (mainly based upon the use of *O*-protected Tfm-GABOB templates) failed, owing to the poor nucleophilicity of the α-Tfm amino group. The final coupling of **34** with Iva–Val–Val–OH (**35**), prepared from commercial H–Val–Val–OH by standard solution-phase technique (82%), was achieved in



Scheme 4. Key: (i) H–Ala–OMe, HATU–HOAt, DMF–TMP; (ii) $LiOH \cdot H_2O$, THF/H_2O; (iii) HATU–HOAt, DMF–TMP; (iv) $Pd(OH)_2/C$, H_2, MeOH; (v) *iso*-$BuCO_2Cl$, NMM, AcOEt, 4 days; (vi) $DMSO/H_2O$, $LiOH \cdot H_2O$.

an epimerization-free manner by using the conditions reported by Bartlett for the synthesis of a phosphorus-containing analogue of pepstatin [31], which provided the stereochemically pure pentapeptide **36**, that was hydrolyzed with LiOH in excellent yield to the final target pepstatin analogue **37**.

Unfortunately, up to a concentration of 150 μM, compound **37** did not show any inhibition of the proteolytic activity of HIV-1 protease. In contrast, although it is well known that natural pepstatin is a very weak (if any) inhibitor of matrix metalloproteinases (MMPs) [32–34], we found that compounds **37**, **30** and **31** reduced MMP-9 (gelatinase B) total potential gelatinolytic capacity in gelatin-zymography. This effect is probably due to an inhibitory effect of the compounds on proteinase release from cells. However, our data on the secreted proteinase suggest that part of this effect is consequent to a direct inhibition of proteinase activity. In fact, compounds **28**, **29**, **30** at 10 μM significantly inhibited MMP-9 gelatinolytic capacity (about 40% inhibition). Compound **29** was tested also at the concentration of 100 μM, and a reduction of MMP-9 activity up to 60% was observed.

3. Trifluromethyl-retro- and retro-inverso peptides

In the previous section we have evidenced the troubles connected with the use of peptide-drugs, and the need for producing analogues that can overcome the barriers and drawbacks described above, while retaining selected activity. Two of the most popular strategies to modify parent peptides **38** (Fig. 3) are: (a) to replace a peptide bond with a surrogate unit X, which is usually symbolized as ψ(X) [35–37]; (b) to reverse all or some of the peptide bonds (NHCO instead of CONH) giving rise to the so called retro-peptides or partially-modified retro-peptides **39** [38], respectively, according to the seminal recipe by Goodman and Chorev [39,40]. When the stereochemistry of one or more AAs of the reversed segment is inverted, the resulting pseudo-peptide is termed as retro-inverso **40**. A malonic unit is classically incorporated to provide partially-modified retro-peptides, while the direction can be restored incorporating a *gem*-diaminoalkyl unit. We proposed a novel class of pseudo-peptides **41** having a $\psi[NHCH(CF_3)]$ as a possible mimic of the classical ψ(NHCO) unit featured by retro-peptides [41]. This surrogate is expected to be stable towards proteolytic degradation, *iso*-polar with the NHCO unit, and eventually the stereo-electronically demanding CF_3 might introduce some conformational constraint, thus limiting the number of stable conformational isomers, as well as modify the binding properties acting as a coordinative site with

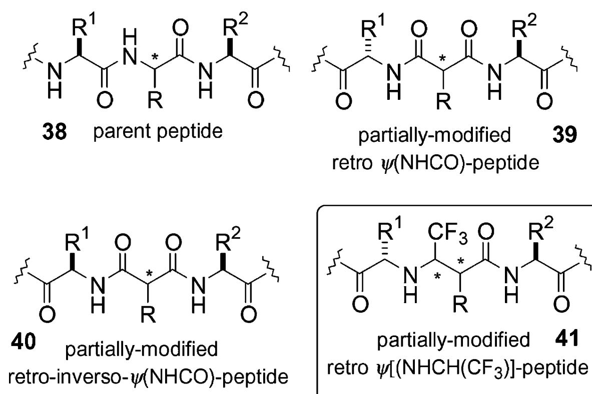
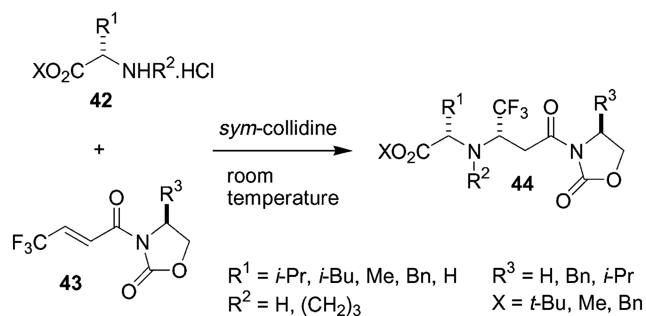


Fig. 3.



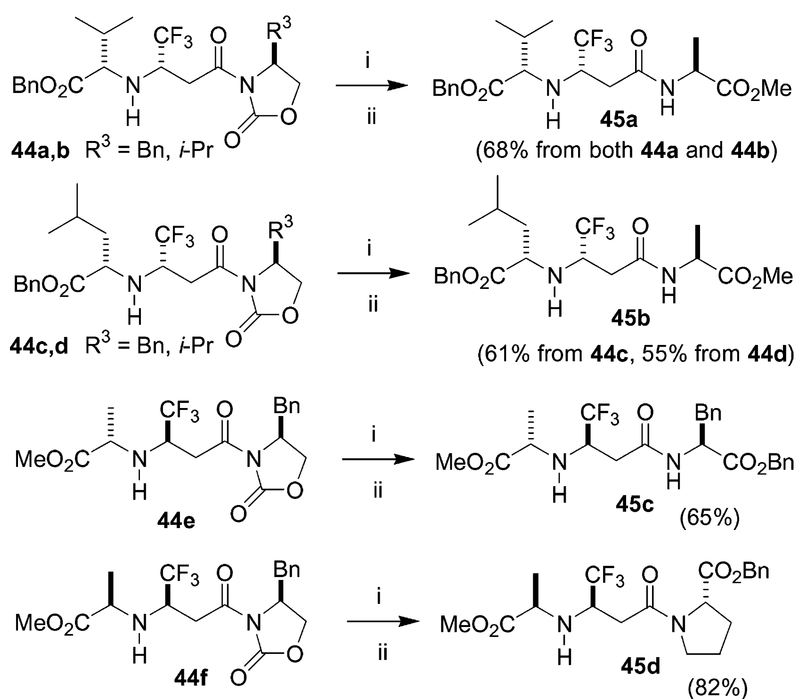
Scheme 5.

enzymes or receptor subsites, or as a hydrogen bond acceptor. Moreover, one of the most serious drawbacks of retropeptides **39** and **40**, that is the configurational instability of the bis(amidated) malonyl centre (when R $\neq$ H), in **41** should be suppressed.

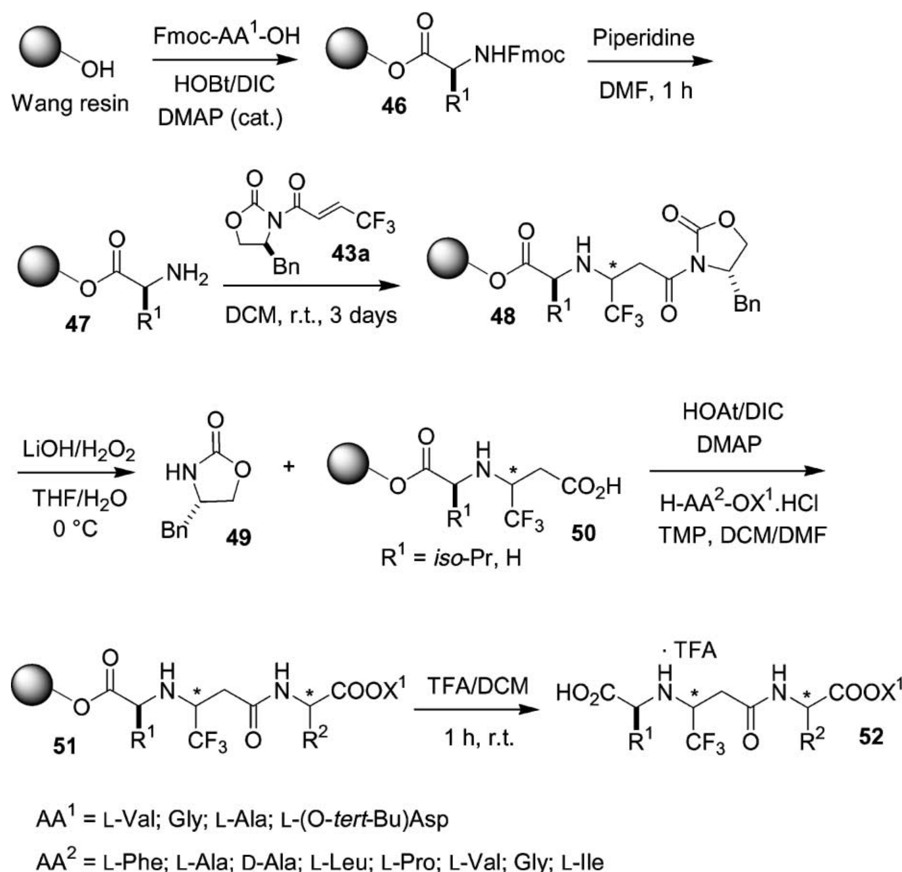
Synthesis of ψ[NHCH(CF_3)]-containing dipeptide units was achieved in excellent yields by conjugate *N*-addition of a series of L-α-amino esters **42** (Scheme 5) to the enantiomerically and geometrically pure Michael acceptors (*S*)-(*E*)-**43**. This methodology is extraordinarily simple and efficient, if one considers that examples of 1,4-additions by chiral α-amino esters to 4-substituted Michael-acceptors are very scarce in the literature [42–44]. The stereochemical outcome of the reaction was studied in detail. The facial diastereoselectivity is mainly controlled by α-amino esters **42**, and the stereo-electronic features of the R^1 side-chain have a remarkable impact on the degree of diastereoselectivity, which follows the trend *iso*-Pr > *iso*-Bu > Me > Bn > H (d.e. up to 78% in the case of *iso*-Pr). Modest d.e. but good yield was obtained with the cyclic α-amino ester H-(L-Pro)-OBn. The R^3 substituent on the oxazolidinone residue had lower effect on the stereoselectivity [78, 72 and 65% d.e. for R^3 = *iso*-Pr, Bn, H, respectively, from H-(L-Val)-OBn], and the steric hindrance of the X group has little influence. The results above can be rationalized if one considers that in the absence of chelating agents *N*-(*E*)-enoyl-oxazolidin-2-ones **43** are known to exist in *s-trans* conformation, with the R^3 substituent being pointed away from the C=C bond, thus exerting little control of the facial selectivity [45–47]. In contrast, the R^1 side-chain of α-amino esters **42** should be spatially close to the forming stereogenic center in the transition state, therefore its influence is much more important. The fact that the stereocontrol improves progressively with decreasing the polarity of the solvent (DCM provides the best results in comparison with acetonitrile, THF, ethanol and DMF) suggests that α-amino esters **42** might react in a well defined, possibly intramolecularly bonded, conformation.

Chemoselective cleavage of the oxazolidinone auxiliary was achieved in 55–82% yields upon treatment of **44a–f** with LiOH/H_2O_2 (Scheme 6). The resulting pseudo-dipeptides having a terminal CO_2H group were purified by FC, then coupled with another α-amino ester. The final retro- and retro-inverso tripeptides **45a–d**, orthogonally protected at the carboxy end-groups and therefore suitable for further selective elongation, were obtained in good yields.

In order to produce combinatorial libraries of ψ[NHCH(CF_3)] pseudo-peptides for biological screening as enzyme inhibitors, we developed an efficient solid-phase approach to these compounds [48]. In the first step (Scheme 7), Wang resin was loaded with Fmoc-α-AAs



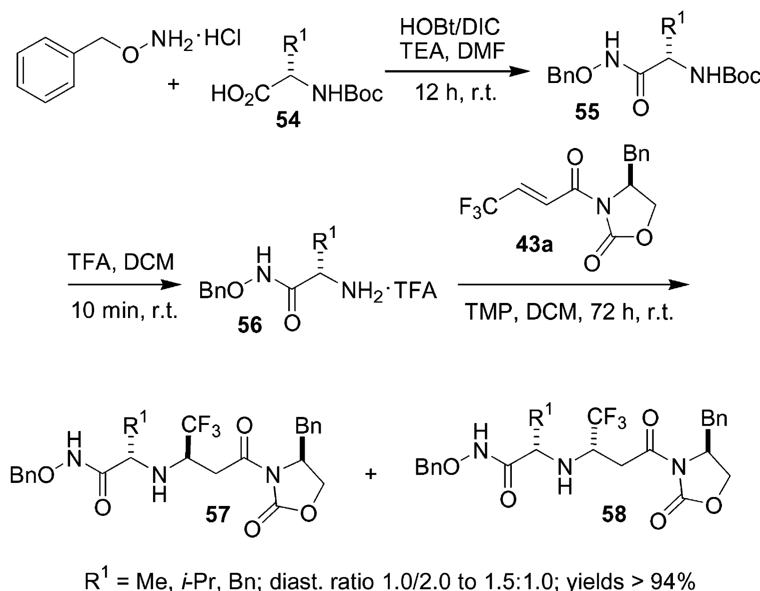
Scheme 6. Key: (i) LiOH/H_2O_2; (ii) HATU–HOAt, *sym*-collidine, DMF,α-amino ester.



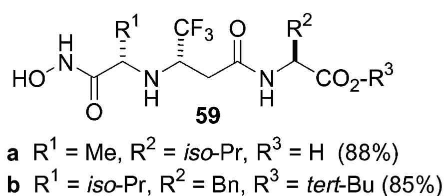
Scheme 7.

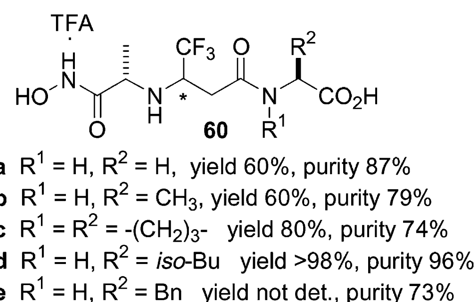
providing the Fmoc-resins **46**, which were *N*-deprotected to **47** according to standard procedures. Next, the resins **47** were submitted to the key 1,4-conjugate addition with 3-(*E*-enoyl)-1,3-oxazolidin-2-one **43a**, which took place efficiently in 3 days at RT affording the resins **48**. To our knowledge, these are the first examples reported in literature of solid-phase intermolecular Michael-type *N*-additions involving α-amino esters and 4-substituted acceptors. The diastereoselectivity is in line with that observed in solution-phase reactions. Exocyclic cleavage of the oxazolidin-2-one **49** from the resins **48** could be achieved with excellent chemoselectivity, without affecting the other hydrolyzable bonds, upon treatment with LiOOH which provided **50**. Coupling of the α-amino esters H–AA^2–OX^1 with the resins **50** was carried out using the diisopropylcarbodiimide (DIC)–1-hydroxy-7-azabenzotriazole (HOAt) system. A representative library of nine retro and retro-inverso resin-bound ψ[NHCH(CF_3)]-peptides **51** was prepared using a parallel synthesizer. Finally, treatment of the resins **51** with TFA produced the release of the target peptidomimetics **52** from the solid support, in good to excellent overall yields and purity in all cases.

Metalloproteases have a key role in a number of serious pathological conditions, such as inflammatory diseases, atherosclerosis and cancers, and their inhibition constitutes a primary therapeutic target [49]. Compounds containing a terminal hydroxamate function are the most potent inhibitors of these enzymes, since the HONHCO– end group is very effective in coordinating the Zn^{2+} cofactor [9]. This explains the current interest in developing novel synthetic routes and novel structural classes of hydroxamate peptidomimetics, possibly using solid-phase/combinatorial techniques which provide ready access to libraries of compounds for fast simultaneous screening, from which the most potent inhibitors are selected [50–55]. Therefore, an efficient solution and solid-phase synthesis of partially-modified (PM) retro-ψ[NHCH(CF_3)]-peptidyl hydroxamates, a novel class of hydroxamates incorporating the [CH(CF_3)CH_2CO] unit, have been developed [56]. Compounds **56** (Scheme 8) were prepared in solution by 1-hydroxybenzotriazole (HOBt)/DIC promoted condensation of *N*-Boc α-AAs **54** with *O*-Bn-hydroxylamine hydrochloride, followed by *N*-Boc-cleavage of the resulting **55** with TFA. The diastereomeric adducts **57** and **58** were formed by conjugate addition of **56** to **43a** for 72 h at RT. These reactions were very clean and high yielding, although low diastereocontrol was achieved in all cases. Comparison of this result with that obtained with the corresponding α-amino esters **42** (see Scheme 5) suggests that AA derived esters and hydroxamates react with Michael acceptors **43** through a different conformation, having a weaker stereodirecting effect. Diastereomerically pure **57** and **58** were isolated by FC.



Scheme 8.





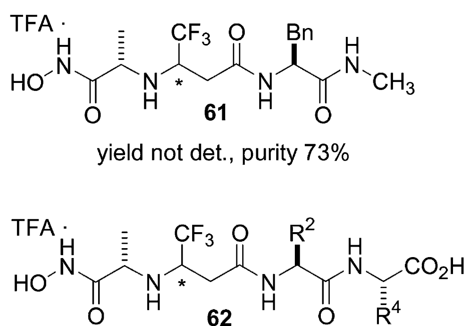


Fig. 4.

Standard exocyclic oxazolidin-2-one cleavage followed coupling to H–(L-Val)–OBn and H–(L-Phe)–*O-tert*-Bu, and finally by catalytic hydrogenolysis of the terminal OBn groups afforded the target hydroxamates **59** (Fig. 4).

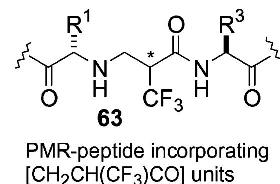
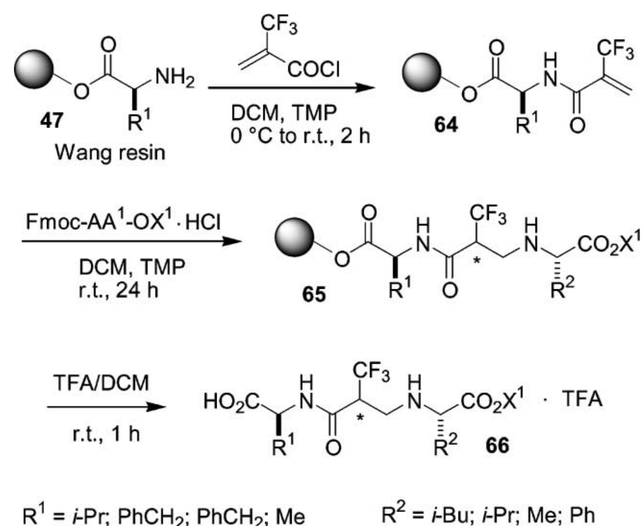


Fig. 5.



Scheme 9.

The method above was also adapted to the solid-phase. Retro-tripeptidyl hydroxamates **60** (Fig. 4) having a CO_2H terminus were obtained in good yields and purity by means of the usual synthetic sequence, starting from an hydroxylamine resin prepared in two steps from commercial Wang resin, according to the method of Floyd [57]. Analogously, we prepared by the same solid-phase method some tripeptidyl hydroxamates having a methylamide terminus (**61**), which is often encountered in metalloprotase inhibitors, and tetrapeptidyl hydroxamates having a CO_2H terminus (**62**).

Very recently, in collaboration with the group of Fustero, we have accomplished a solid-phase synthesis of a novel family of Tfm-substituted PMR-peptides **63** (Fig. 5) incorporating a stereogenic [$CH_2CH(CF_3)CO$] unit [58].

The resins **47** (Scheme 9) were reacted with 2-trifluoromethyl-propenoyl chloride, prepared from the commercially available acid, providing the Tfm-resins **64**, functionalized as chiral Michael acceptors [59]. The crucial Michael *N*-additions were performed by reacting 3 eq. of the appropriate α-amino ester with the resin **64**, producing the desired resins **65** in a very effective manner. As usual, the stereocontrol was progressively higher with increasing the bulk of R^2, spanning from 1.5:1.0 for $R^2 = Me$ to 5.8:1.0 when $R^2 = iso$-Bu. A representative library of the target PMR-peptides **66** was released from the solid support in very good purity by means of TFA.

4. Conclusions

We have shown that a wide range of enantiomerically pure Tfm-containing peptides and pseudopeptides can be synthesized in a stereocontrolled manner. The effectiveness of these methodologies, combined with the successful application of solid-phase techniques, is expected to open up the route to further classes and combinatorial libraries of fluorinated peptidomimetics, allowing for a systematic study of their hitherto unknown conformational and biological properties, which are likely to be extremely intriguing and peculiar owing to the presence of fluorine.

Acknowledgements

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