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# The Trifluoroethylamine Function as Peptide Bond Replacement

Monica Sani, Alessandro Volonterio, and Matteo Zanda*[a]

Peptides play a fundamental role in a number of physiological processes, and can be used as drugs in various therapeutic classes. However, a major drawback of peptides is represented by their low bioavailability, consequently they have to be injected or administered in the form of expensive formulations.[1] For this reason, the design and synthesis of metabolically stable peptide analogues that can either mimic or block the bioactivity of natural peptides or enzymes is an important issue in bioorganic and medicinal chemistry research. The replacement of a scissile peptide bond represents a viable and popular approach in the rational design of peptidomimetics. Peptidomimetics find applications as drugs, in protein engineering and so on. This is evident from the wealth of therapeutically useful peptidomimetic leads incorporating any of the peptide bond surrogates that are currently available.[2] Therefore, within the realm of peptide mimics, the replacement of amide bonds with appropriate functionalities is a classic approach in medicinal chemistry and drug discovery. Some peptide bond surrogates, such as the alkene (–CR=CH–) or fluoroalkene (–CF=CH–) functions, are purely geometric replacements preserving little or none of the capacity of the native peptide bond to undertake electrostatic interactions or hydrogen-bonding with the receptor. Other amide replacements are known which retain the geometry of the amide bond or maintain the hydrogen bond-accepting properties of the amide, such as the ketomethylene (–$COCH_2$–) or depsipeptide (–$CO_2$–) functions. However, there are a few functional groups, which are capable of preserving the hydrogen bond-donating properties of the amide. Among them, one can mention sulfonamides (–$SO_2$–NH–), anilines, secondary alcohols, hydrazines, and certain heterocycles. How to minimize the basicity of an NH donor, so that a $NH_2^+$ moiety is not formed at physiological pH, is the main issue for identifying a truly effective NH amide replacement. In fact, ammonium ions are poorly tolerated deep in the active site of a protein where binding interactions cannot compensate for the energetic cost of desolvation. This is a serious drawback of popular peptide bond surrogates, such as the methyleneamino (–$CH_2$–NH-) function. Currently, a powerful new strategy is emerging for replacing the peptide bond with a very effective surrogate: the stereogenic trifluoroethylamine function. Indeed, a trifluoroethyl group can replace the carbonyl of an amide and generate a metabolically stable, poorly basic amine that maintains the excellent hydrogen bond of an amide. This strategy was first proposed by our research group, and was used to replace both a glycine amide bond and a malonamide of a partially modified retro (PMR) peptide.[3] The main properties featured by the trifluoroethylamino group are: 1) very low NH basicity, 2) a $CH(CF_3)NHCH$ backbone angle close to 120°, 3) a C–$CF_3$ bond substantially isopolar with the C=O, and 4) structural analogy with the tetrahedral proteolytic transition state. Furthermore, the $sp^3$ hybridization of all the atoms forming the stereogenic trifluoroethylamine moiety is expected to allow a better orientation of the atoms in the receptors' active sites, thus optimizing the energetically favorable interactions (hydrogen-bonds, van der Waals, hydrophobic, etc.). With this in mind, in 2000 we published the first examples of peptidomimetic structures incorporating a trifluoroethylamine unit replacing the retropeptide bond.[4] In that first work, we presented a number of PMR and partially modified retro-inverso (PMRI) peptides (Figure 1) in which one of the amide bonds involving the conventional retropeptidic malonic unit was replaced by a trifluoroethylamine function. The synthetic protocol for the preparation of trifluoroethylamine-based partially modified retropeptides was based on a key aza-Michael addition of α-amino acid esters **1** and a fluorinated enoyl oxazolidin-2-one **2** (Scheme 1). The solvent has a strong influence on stereoselectivity, and the best results were obtained in dichloromethane (DCM), whereas a substantial drop of *de* was observed for the major diastereomers **3**

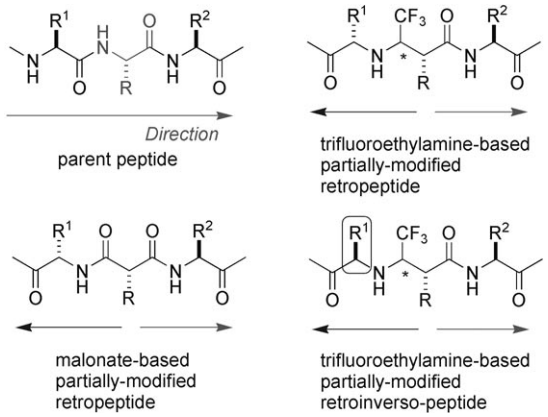


**Figure 1.** Trifluoroethylamine-based partially modified retro and retroinverso peptides.

[a] *Dr. M. Sani, Dr. A. Volonterio, Dr. M. Zanda*
*C.N.R. Istituto di Chimica del Riconoscimento Molecolare and Dipartimento C.M.I.C., Politecnico di Milano Via Mancinelli 7, 20131 Milan (Italy)*
*Fax: (+39) 0223993080*
*E-mail: matteo.zanda@polimi.it*

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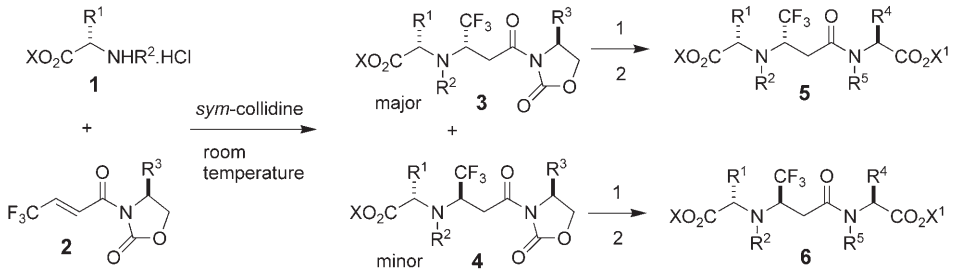
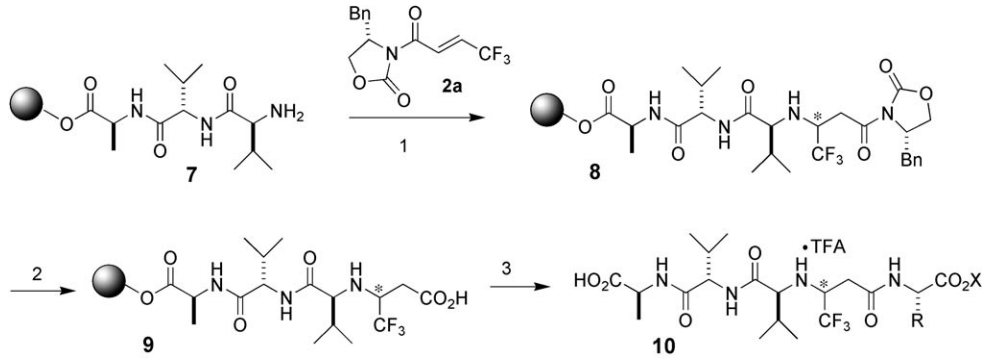


**Scheme 1.** Aza-Michael reaction with amino-ester nucleophiles. Key: 1) LiOH, $H_2O_2$; 2) HATU/HOAt, *sym*-collidine, DMF, α-amino ester.

with more polar solvents such as ethanol, acetonitrile, THF, DMF, or mixtures thereof. The base also plays an active role, as demonstrated by the fact that the use of DABCO, instead of *sym*-collidine (TMP), accelerates the reaction but slightly lowers the *de* of the products **3**. We could also demonstrate that the facial diastereoselectivity of these reactions is mainly controlled by the nucleophiles **1** rather than by the oxazolidin-2-one acceptors **2**. The degree of diastereoselectivity, followed the trend $R^1 = i\text{Pr} > i\text{Bu} > \text{Me} > \text{Bn} > \text{H}$. Modest *de* was obtained when **1** is the cyclic α-amino ester L-Pro-OBn. On the other hand, the $R^3$ substituent on the stereocenter of the oxazolidinone **2** had a lower effect on the stereoselectivity. No meaningful effect was exerted by the X group of the nucleophiles **1**. The stereocontrol of this process was therefore maximized using DCM as solvent, TMP as base, and nucleophiles having a bulky $R^1$ chain, such as Val, leading to *de* up to 78%. The method is viable for preparing longer PMR and PMRI Ψ[NHCH-$(CF_3)$]Gly-peptidyl-oxazolidinones by using unprotected *N*-terminal peptides as nucleophiles in the aza-Michael reactions, that occurred with excellent yields and good stereocontrol. The chemoselective cleavage of the oxazolidinone auxiliary from **3**, **4** was achieved in 55–82% yields upon treatment with $LiOH/H_2O_2$. The resulting pseudopeptides having a terminal $CO_2H$ group were coupled with another α-amino ester. The final diastereomeric PMR and PMRI tripeptides **5**, **6**, orthogonally protected at the carboxy endgroups, and therefore suitable for further selective elongation, were obtained in quantitative yields, often as solid materials. The parallel solid-phase synthesis of small libraries of PMR-Ψ[NHCH$(CF_3)$]Gly tri-, tetra-, and pentapeptides, which should be applicable in the preparation of wider libraries of PMR-Ψ[NHCH$(CF_3)$] polypeptides for high-throughput biological screening, was also developed.[5] As an example, in Scheme 2 the synthesis of PMR-Ψ[NHCH$(CF_3)$]Gly pentapeptides **10** is described. The tripeptide resin H-Val-Val-Ala-OWang **7** was prepared by Fmoc-chemistry and subjected to the conjugate addition with a fluorinated enoyl oxazolidin-2-one **2a**, which took place very effectively in three days at RT. A 10:1 mixture of aza-Michael diastereomeric adducts **8** was formed. In general these aza-Michael reactions in solid-phase occurred with diastereocontrols comparable with those in solution (see above). Interestingly, in the case of tripeptides **7** as polymer supported nucleophiles, we observed the highest diastereoselection as compared with the conjugate additions of H-Val-OWang and H-Val-Gly-OWang (7:1 and 4.5:1 mixtures of diastereomers, respectively). This shows that additional stereocenters, even in remote positions of the nucleophile, can have a strong influence on the stereochemical outcome of the conjugate additions. The resulting resin **8** was chemoselectively hydrolyzed at the C terminus by treatment with lithium hydroperoxide generated in situ. The resulting pseudotetrapeptide resin **9** was coupled (HOAt/DIC) with different α-amino acid esters generating, after release from the resin, the final PMR-Ψ[NHCH-$(CF_3)$]Gly pentapeptides **10** with very good overall yields and purity. These fluorinated retropeptides were also functionalized as terminal hydroxamic acids and synthesized by means of solid-phase methods. The conformational features of PMR Ψ-[NHCH$(CF_3)$]Gly-peptides were studied in detail.[6] It has been shown that very simple PMR-peptides, such as the triamide **11** (Figure 2), adopt turnlike nine-membered folding patterns with an intramolecular N–H···O=C hydrogen bond in the solid state, as well as in low-polarity solvent solutions.[7] Racemic Ψ[NHCH-$(CF_3)$]-diamide **12** (Figure 2) was synthesized in good overall yield by means of the standard protocol (see Scheme 1). $^1$H NMR spectroscopy supported by MD calculations showed



**Scheme 2.** Solid-phase synthesis of PMR-Ψ[NHCH$(CF_3)$]Gly pentapeptides. Key: 1) DCM, RT, 3 days; 2) $LiOH/H_2O_2$, $THF/H_2O$, 0 °C; 3) H-AA-OX·HCl, HOAt/DIC, TMP, DMAP (cat), then TFA/DCM, 1 h, RT.

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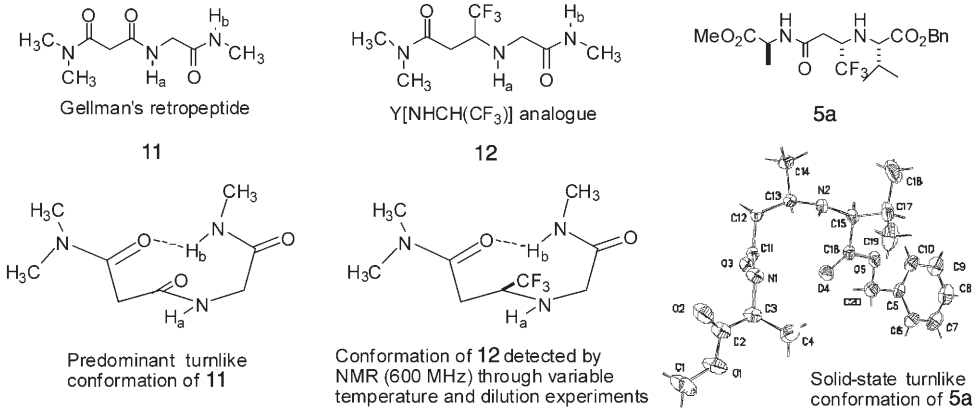



**Figure 2.** Solution and solid-state conformation of some PMR Ψ[NHCH($CF_3$)]Gly-peptides.

the stability of turnlike conformations for **12** in a weakly hydrogen bonding solvent, such as $CDCl_3$, which are comparable to that of parent malonyl-based retropeptides **11**. Similar turnlike conformations were found in the solid-state, as demonstrated by X-ray diffraction studies of several Ψ[NHCH($CF_3$)]Gly-peptides, such as **5a** (Figure 2). This very interesting conformational behavior is a likely consequence of two main factors: 1) severe torsional restrictions about $sp^3$ bonds in the [CO–$CH_2$–CH($CF_3$)–NH–CH(R)–CO] module, which is biased by the stereoelectronically demanding $CF_3$ group and the R side chain; 2) formation of nine-membered intramolecularly hydrogen-bonded rings, which have been clearly detected both in $CHCl_3$ solution and in some crystal structures. The first factor seems to be more important, as turnlike conformations were found in the solid-state even in the absence of intramolecular hydrogen bonding. The relative configuration of the –C*H-($CF_3$)NHC*H(R)– stereogenic centers was found to have a major effect on the stability of the turnlike conformation, which seems to require a *syn* stereochemistry, such as **5** (Scheme 1), whereas the diastereomers **6** investigated so far did not show the same conformational properties. Next, we reported a significant advancement of our project, consisting in the stereocontrolled synthesis of a new class of peptidomimetics, much closer to natural peptides, having a fluoroalkyl backbone modification: Ψ[CH($CF_3$)NH]Gly-Peptides **13** (Figure 3, R = H).[8] In this case, the trifluoroethylamine function replaces a native peptidic amide-bond [CO–NH]. The stereocontrolled synthesis of these new peptidomimetics is based on another key aza-Michael reaction involving 3,3,3-trifluoro-1-nitropropene **14** (Scheme 3) and an array of α-amino esters, generated in situ from the hydrochlorides **1** with a base. The reactions took place almost instantaneously at RT, affording the diastereomeric α′-Tfm-β′-nitro α-amino esters **15** (major)

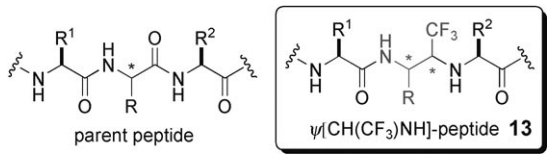
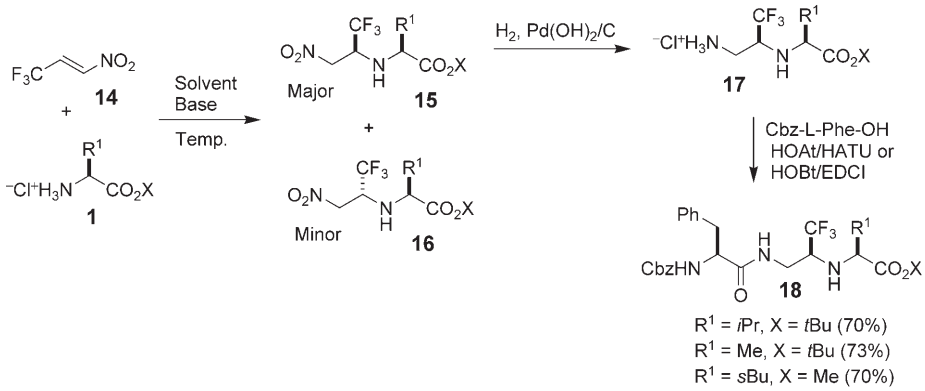



**Figure 3.** Peptidomimetics **13** incorporating a trifluoroethylamine replacing the native peptide unit.

and **16** (minor) under kinetic control (Scheme 3). The diastereoselectivity of the process was studied in detail. We found that it depends mainly on four reaction parameters: 1) base, 2) solvent, 3) stoichiometry of the base, and 4) R side chain of **1**. Concerning the base, the best stereocontrol (63% *de* using L-Val esters as nucleophiles) was achieved with DIPEA, whereas $NaHCO_3$, TMP, and DABCO gave modest results. As observed for other aza-Michael reactions (see above), low-polarity solvents provided remarkably higher diastereocontrol. Thus, toluene afforded **15** with 84% *de*, whereas DCM and THF afforded modest *de* values. Quite surprisingly, intermediate results were observed using apolar $CCl_4$. Even more surprisingly, the stoichiometry of DIPEA was also found to have a profound effect on the stereocontrol. The optimum amount was found to be 1.1 equiv (as used in the experiments cited above). In the ab-




**Scheme 3.** The aza-Michael reaction to give the Ψ[NHCH($CF_3$)]Gly-peptide backbone, and subsequent elaboration into the Ψ[CH($CF_3$)NH]Gly-Peptides **18**.

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sence of free DIPEA the *de* dropped dramatically. Accordingly, a progressive decrease of stereoselectivity was observed by increasing the amount of DIPEA from 1.1 to 1.7 equiv, whereas little variation occurred beyond this quantity. Other bases, such as TMP and $NaHCO_3$, did not feature the same "stoichiometry-effect" affording comparable *de* values upon changing the number of equivalents used. The effect of the R side chain of **1** was in line with expectations. In fact, the highest *de* values were observed with bulky R groups (*i*Pr, *s*Bu) whereas lower degrees of stereocontrol where observed with R = Me, Bn, etc. Room temperature was found to be essential to achieve high yields of **15** and **16**, whereas, surprisingly, at lower temperatures (for example −40 or −70 °C) complex mixtures of products were obtained. All the experimental evidence above suggests that these aza-Michael reactions occur through a tight, polar, termolecular transition state (TS), involving **1**, **14**, and DIPEA, which appears to play a fundamental catalytic role. Polar solvents, and the presence of more than one molecule of DIPEA, may disrupt this TS, thus lowering the stereocontrol. Elaboration of the major adducts **15a**–**c** into the target Ψ[CH-($CF_3$)NH]Gly-peptides **18a**–**c** is shown in Scheme 3. The nitro group of **15a**–**c** was hydrogenated to an amino group using the Pearlman's catalyst, and the resulting diamino compounds were trapped as hydrochlorides **17a**–**c** and submitted without purification to coupling with Cbz-L-Phe-OH affording the Ψ[CH($CF_3$)NH]Gly-tripeptides **18a**–**c** in good overall yields. To validate the scope of this synthetic methodology in the preparation of more complex Ψ[CH($CF_3$)NH]Gly-peptides, we have recently completed the synthesis of the tetrapeptides **19**, **20**, and their epimers at the trifluoroethylamine stereocenter (Figure 4).[9] The latter is an interesting model for conformational studies because some of the analogues incorporating a

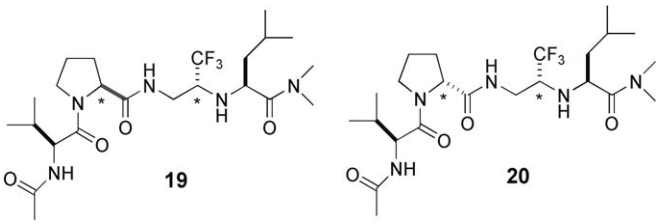


**Figure 4.** Complex models of Ψ[CH($CF_3$)NH]Gly-tetrapeptides.

natural Gly have been shown to assume very stable and highly populated β-hairpin conformations, depending on the configuration of the Pro residue.[10] In the case of the two peptides incorporating a L-Pro, NMR experiments and MD calculations indicated a population of conformers which adopt folded β turns structures forming a 10 membered ring showing a hydrogen bond between aminic proton $NH_c$ of L-Leu and the CO of L-Val (Figure 5).

Analogously, NMR studies and MD calculations on the peptide **20** incorporating D-Pro (Figure 6) showed structures forming a 10-membered hydrogen bond between the aminic

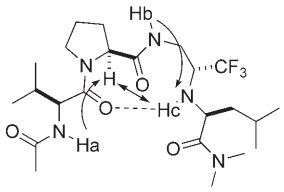


**Figure 5.** Schematic representation of the structure of **19** in $CDCl_3$. The arrows indicate the diagnostic NOE interactions. No NOEs were found between terminal $CH_3$ and $NH_a$ with the N-methyl terminal groups.

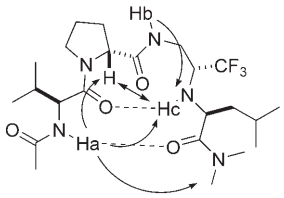


**Figure 6.** Schematic representation of the structure of **20** in $CDCl_3$. The arrows indicate the diagnostic NOE interactions. NOEs were found between $NH_a$ an $NH_c$ and with $NH_a$ and the N-methyl terminal group.

proton $NH_c$ of L-Leu and the CO of L-Val and a second hydrogen bond between $NH_a$ and the CO of L-Leu. This evidence proved that the change of the stereochemistry at the Pro induces a natural folding towards a β hairpin conformation. It is noteworthy that a different configuration of the trifluoromethylamino group for peptides **19** and **20** does not affect their conformation, as evidenced by NMR studies and MD calculations on the trifluoroethylamine epimers of **19** and **20**. The fact that the aminic proton $NH_c$ of peptidomimetic **19**,**20** is involved in an intramolecular hydrogen bond stabilizing secondary structures is remarkable, as it further validates the concept that the trifluoroethylamino function is an effective surrogate for the peptide bond. Recently we performed the synthesis of the Ψ[NHCH($CF_3$)] analogue **24** of retrothiorphan (Scheme 4),[11] which is a potent and selective inhibitor of the metalloproteinase NEP (neutral endopeptidase) that does not inhibit another zinc proteinase ACE (angiotensin converting enzyme), which has a key role in the control of blood pressure.[12] As angiotensin I is a substrate for both ACE and NEP, it is clear that selective inhibition of the two enzymes can have therapeutic relevance. It is worth noting that the aza-Michael reaction of the fluorinated oxazolidin-2-one acceptor **2b** with several different β-amino-alcohols as nucleophiles invariably occurred with low diastereocontrol. The aza-Michael adduct **21**, obtained according to the general strategy explained above, was converted into the target product **24** in three steps and moderate overall yield (see Scheme 4). The other stereoisomers were also prepared. All the Ψ[NHCH($CF_3$)]-retrothiorphan diastereomers **24** were subjected to biological tests (fluorometric assay) to evaluate their inhibitory capacity toward neutral endopeptidase 24.11 (NEP), showing $IC_{50}$ values several orders of magnitude higher than thiorphan, with $K_i$ values over 4 μM (for reference compound: $IC_{50}$ = 5.06 nM, $K_i$ = 2.53 nM). Moreover, the comparison of the results obtained for the new

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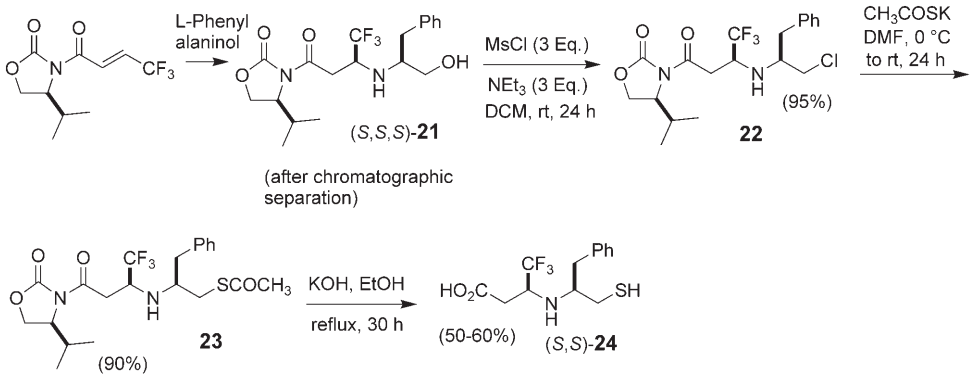


**Scheme 4.** Synthesis of stereochemically pure Ψ[NHCH($CF_3$)]-retro-thiorphan **24**.

Ψ[NHCH($CF_3$)] compound **24** and its stereoisomers with the data reported for (*R*) and (*S*)-retrothiorphan ($K_i = 2.3$ nM and 210 nM, respectively)[13] confirmed the loss of the NEP inhibition capacity. Despite the negative result above, this innovative "trifluoroethylamine replacement" strategy has recently found the first validation in drug discovery. In fact, the Merck-Frosst center in Montréal (Canada) has published[14] that trifluoroethylamines can be successfully used as amide replacements in highly potent and metabolically stable inhibitors of cathepsin K, a cysteine proteinase thought to be responsible for degradation of type I collagen in osteoclastic bone resorption that represents a highly promising target for the therapy of osteoporosis. Thus, the trifluoroethylamine compound **25** (Figure 7) was found to be an extremely potent (with an $IC_{50}$ value in the picomolar range) and selective inhibitor of cathepsin K. It is interesting that both potency and selectivity were increased with respect to the corresponding dipeptide analogue **26** (having a natural C=O unit instead of the trifluoroethylamine mimic), and in comparison to the epimeric trifluoroethylamine **27**, confirming the importance of the stereochemistry in these peptidomimetics. Next, the same group reported that another trifluoroethylamine compound **28** (Figure 8),[15] showing subnanomolar $IC_{50}$ values against cathepsin K and excellent selectivity toward other cathepsins, displays excellent pharmacokinetics and efficacy in an appropriate rhesus monkey model. These findings support hopes that xenobiotic, exclusively man-made trifluoroethylamines linkages as peptide bond replacements could actually become drugs in the near future. Compound **28** was synthesized by stereoselective arylation of an imine (**30**) derived from fluoral and suitably protected (*S*)-leucinol **29**, followed by Suzuki coupling of the resulting *p*-Br-derivative **31**, oxidation of the primary β-amino-alcohol **32** to the α-amino acid **33**, and condensation of the latter with aminoacetonitrile (Scheme 5). It is worth noting that the trifluoromethyl group does not make any lipophilic interactions with the enzyme, as demonstrated by an X-ray structure of a complex between one of these trifluoroethylamines and cathepsin K, but rather is directed away from the active site, into water.[15] A very effective and general approach to both epimers of aryl trifluoroethylamine

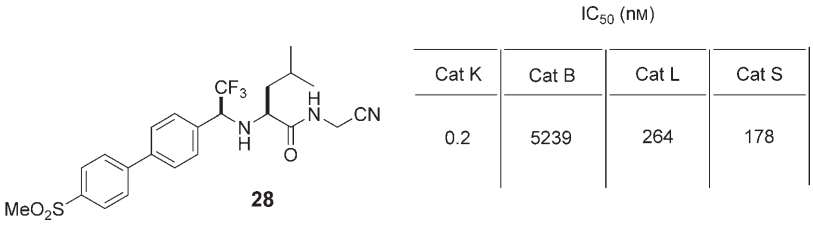


**Figure 8.** Metabolically stable and highly potent Merck-Frosst's cathepsin K inhibitor.

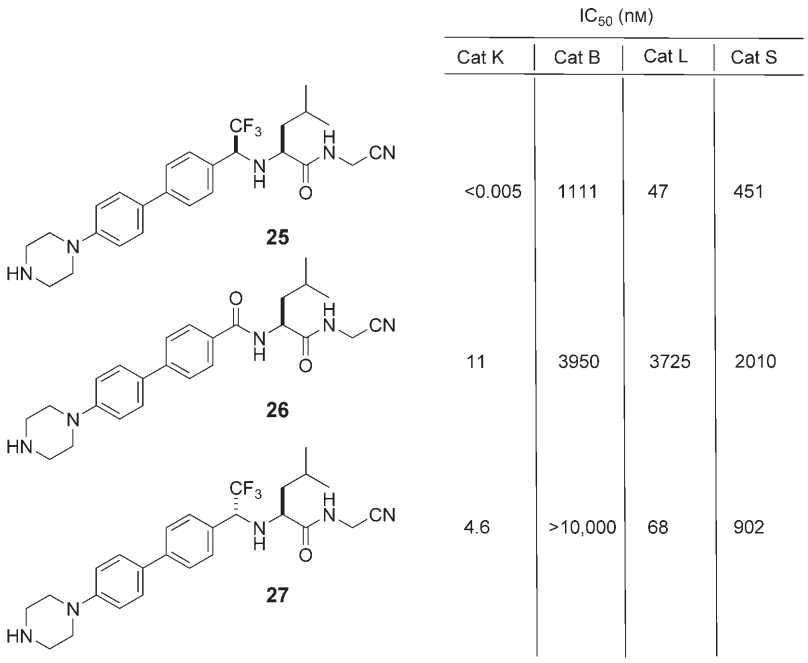


**Figure 7.** Merck-Frosst's trifluoroethylamine inhibitor of Cathepsin K and analogues.

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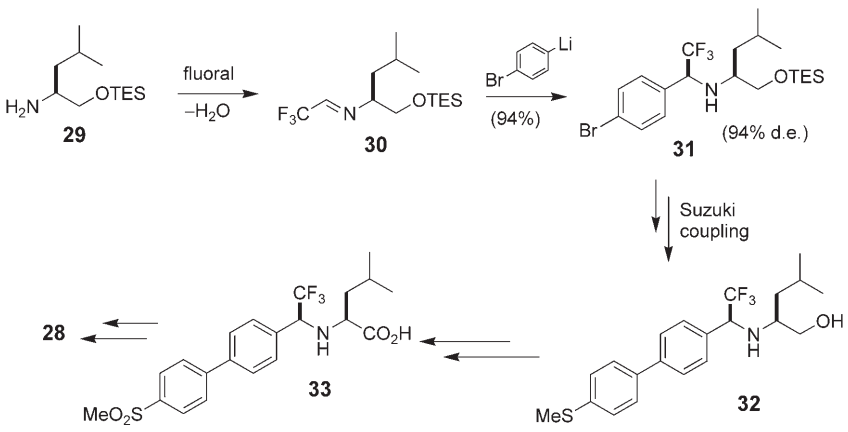



**Scheme 5.** Merck-Frosst's synthesis of the cathepsin K inhibitor **28**.

compounds was very recently reported by Merck-Frosst process chemists.[16] The key imines **34** derived from trifluoroacetophenones and α-amino acid esters were obtained by base-promoted condensation, according to the pathway portrayed in Scheme 6. Interestingly, the acid-promoted version was unsuccessful leading to extended decomposition. Upon treatment of the ketimines **34** with a nonchelating borohydride ($NaBH_4$), the (*R*)-epimer at the $CF_3$-substituted stereocenter **35** was obtained with moderate to excellent control, whereas the (*S*)-epimer **36** was produced with generally good stereocontrol by action of a chelating borohydride such as $Zn(BH_4)_2$. Another route to the aryl trifluoroethylamines was described by another Merck-Frosst group (Scheme 7).[17] In this case, *tert*-butylsulfinimines **37** derived from fluoral hemiacetals were used as electrophiles with aryllithium derivatives, affording the target (*S*)-trifluoroethylamines **38** with excellent stereocontrol, albeit with moderate yields. Less favorable results were achieved by using aryl Grignard reagents, which produced the same (*S*)-configured products **38** but with a lower degree of stereocontrol. Very recently, a report by Celera-Genomics disclosed the use of molecules containing the trifluoroethylamine amide replacement as a key function in cathepsin S inhibitors having nanomolar potency, such as **39** (Figure 9).[18] The body of experimental results listed above suggests important considerations for a successful use of the trifluoroethylamine function as a peptide/retropeptide bond mimic (Figure 10). When the amide or peptide bond to be replaced by the trifluoroethylamine unit is one of the reasons for the low bioavailability of the parent unfluorinated molecule, this strategy can be highly successful. Indeed the trifluoroethylamine unit seems to have high metabolic stability. The trifluoromethyl group, contrary to the carbonyl oxygen, is a weak hydrogen-bond acceptor.[19] The trifluoroethylamine function can be therefore an effective peptide bond replacement only if the carbonyl group of the original ligand amide/peptide-bond is not involved in essential hydrogen-bonding with the receptor. This could explain the dramatic drop of inhibitory activity observed with retrothiorphan analogues (**24**), which might be due to the fact that the retropeptidic carbonyl group of the unfluorinated retrothiorphan is known to be involved in critical interactions with the active site of NEP as hy-

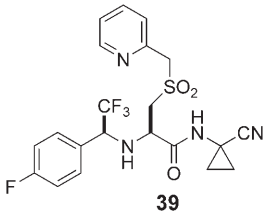
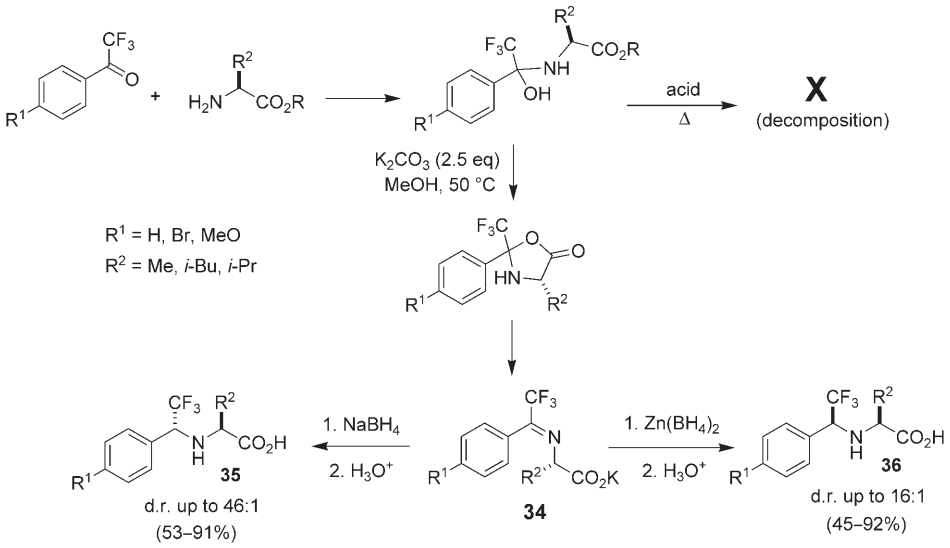
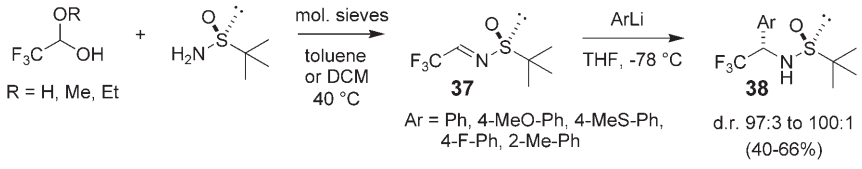



**Figure 9.** Celera-Genomics inhibitor of cathepsin S.




**Scheme 6.** Stereoselective synthesis of both epimers of aryl trifluoroethylamine compounds.




**Scheme 7.** Stereoselective synthesis of aryl trifluoroethylamines via chiral sulfinimines.

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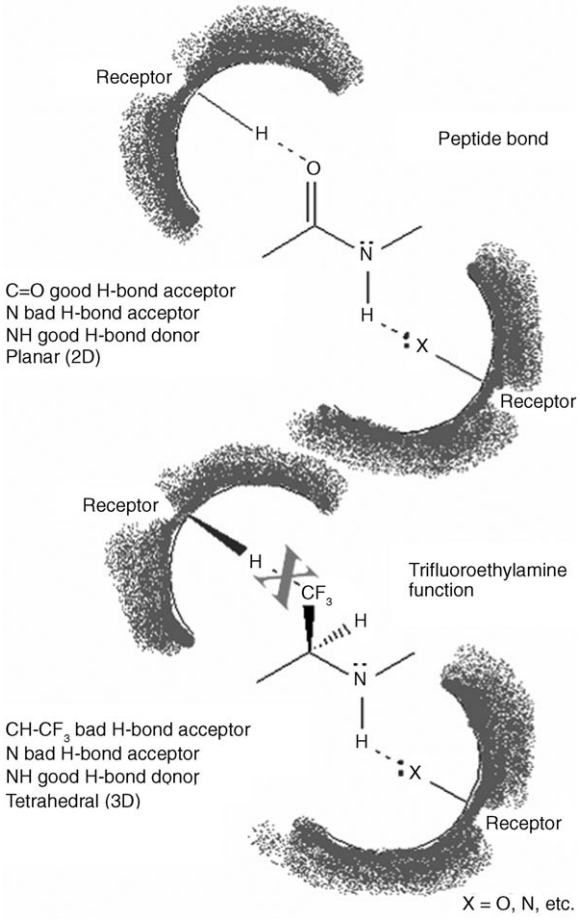


**Figure 10.** Comparison between the peptide (amide) bond and the trifluoroethylamine function.

drogen bond acceptor.[20] The NH of the trifluoroethylamine unit is a good hydrogen-bond donor, because of the strong electron withdrawing effect exerted by the $CF_3$ group, and could always be considered a good mimic of a peptidic NH. The $sp^3$ N atom of the trifluoroethylamine function is a bad hydrogen-bond acceptor and has very little Lewis basicity, in close analogy with the peptide bond. The geometry and spatial orientations of the interactions (including hydrogen-bonds) between the original planar amide/peptide-moiety and the receptor can be optimized by the $sp^3$ tetrahedral configuration of the trifluoroethylamine unit. The trifluoromethyl group is thus important in neutralizing the amine function, generating a poorly basic amine having remarkable similarity with the NH of an amide/peptide group. In fact, the $pK_a$ of protonated compounds such as **25**–**28** (Figures 7 and 8) was reported to be lower than 1.5.[21] However, the role of the $CF_3$ is also extremely important as this sterically large group can impose a steric constraint to the neighboring substituents, forcing them to adopt an orientation which corresponds to the biologically active conformation.[21] In addition, it has been shown that cathepsin K inhibitors incorporating the poorly basic trifluoroethylamine peptide-bond replacement are not affected by problems related with lysosomotropism, despite the fact that this physical property might in fact lead to improved pharmacokinetic properties: a drug that is sequestered in the lysosomes will have reduced exposure to hepatic cytochrome P450 mono-oxygenases, thus allowing the lysosome to act as a depot that will release the drug slowly and result in a long terminal half-life. However, cysteine cathepsins can also be active as secreted and cell membrane associated enzymes. In these cases, no effect of lysosomotropism on inhibitor potency may be expected.[21] In conclusion, the trifluoroethylamino function is a very promising peptide- and amide-bond replacement that will likely find extensive application in medicinal chemistry and drug discovery.[22]

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