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Conjugated additions of amines and β-amino alcohols to trifluorocrotonic acid derivatives: synthesis of ψ[NHCH(CF_3)]-retro-thiorphan

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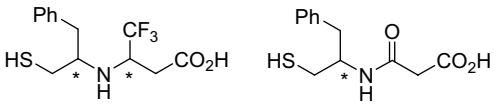
Abstract—The aza-Michael addition of amines and β-amino-alcohols to trifluorocrotonic acid derivatives is described. The reactions occurred in good to excellent yields, but low stereocontrol. The title reaction has been used as a key step for the synthesis of trifluoroethylamine mimics of retro-thiorphan, which showed low inhibitory activity of NEP (constant inhibition at the molar level).

Incorporation of a trifluoromethyl group into peptide and protein structures is emerging as a highly attractive strategy for improving biological activity and modifying structural properties of these molecules.[1] Of particular interest is the replacement of a peptide or amide bond [CONH] with trifluoroethylamine units, to provide the so called ψ[CH(CF_3)NH] isosteres.[2] This strategy, that was originally proposed by our group,[3] has recently found important applications in the development of highly potent and metabolically stable inhibitors of cathepsin K for the therapy of osteoporosis,[4] and of cathepsin S for the therapy of several autoimmune-based inflammatory diseases.[5] Along with the replacement of the native peptide bond,[3c] the trifluoroethylamine unit was also proposed as a surrogate of the retro-peptide bond [NHCO],[6] thus giving rise to ψ[NHCH(CF_3)] isosteres.[3a,b] To this end, we described the synthesis of partially modified ψ[NHCH(CF_3)]-retro-peptides by aza-Michael addition of α-amino acid esters to chiral *N*-trifluorocrotonoyl-oxazolidin-2-ones. However, the use of different nucleophiles, such as amines and amino-alcohols, has not been investigated yet, despite the potential use of such strategy to achieve a straightforward entry in to highly valuable trifluoroethylamine analogues of biologically active molecules.

One of the possible targets is the ψ[NHCH(CF_3)] analogue **1** of retro-thiorphan (**2**) (Fig. 1), which is a potent and selective inhibitor of the metalloproteinase NEP (neutral endopeptidase) sparing another zinc proteinase ACE (angiotensin converting enzyme), which has a key role in the control of blood pressure.[7]

The *N*-trifluorocrotonoyl-oxazolidin-2-one **3** (Scheme 1) was prepared as described in the literature.[3a,b] First experiments carried out by simply mixing the acceptor **3** and benzylamine **4a** (2 equiv) in DCM at rt (the standard procedure used for the aza-Michael additions of α-amino-acid esters to acceptors like **3**)[3a,b] were surprisingly disappointing, since the main product, obtained in modest yields along with several unidentified



1 Retro-thiorphan **2**

Figure 1. Retro-thiorphan (**2**) and ψ[NHCH(CF_3)]-retro-thiorphan (**1**).

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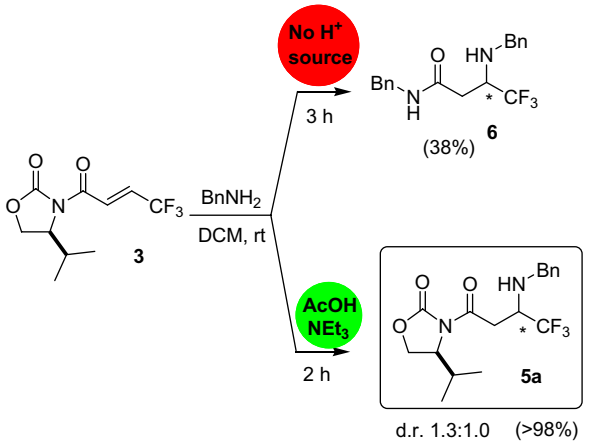
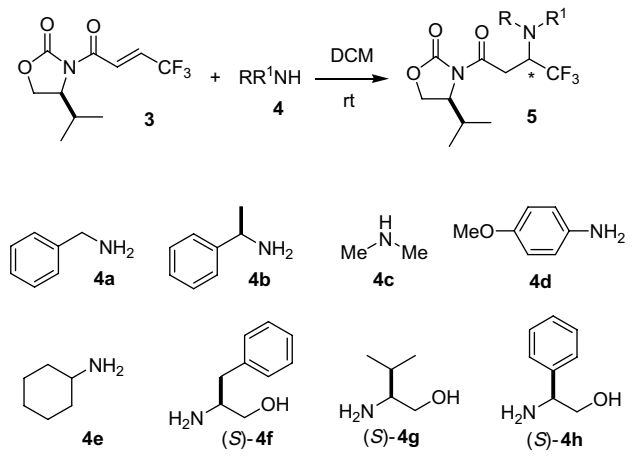


Scheme 1. Set-up of the conditions for the aza-Michael reaction.



Scheme 2. The conjugate additions.

by-products, was amide **6**. We next performed the reaction of **3** with **4a** under conditions of mild acid catalysis, namely in the presence of a weak protic acid like triethylammonium acetate (2 equiv), generated in situ from triethylamine and acetic acid (Table 1, entry 1). Gratifyingly, **5a** was obtained in 2 h at rt in nearly quantitative yields, although with a very low diastereocontrol.[8]

Treatment of adduct **5a** with benzylamine **4a** gave rise to exocyclic cleavage of the oxazolidin-2-one auxiliary, affording amide **6** in 75% yield. However, the reaction was very slow (48 h), suggesting that the observed formation of **6** from **3** and **4a** in the absence of acid catalysis (Scheme 1) could take place through other pathways as well.[9]

An array of aliphatic and aromatic amines, as well as β-amino-alcohols **4** (Scheme 2) was used for exploring scope and limits of the aza-Michael reaction with acceptor **3**. In order to improve the stereocontrol of the process observed with **4a**, the use of a chiral proton source (Table 1, entry 11) and many different Lewis acids (entries 12–15) was attempted, but we were unable to achieve significantly better results in terms of diastereoselectivity, although the use of $MgClO_4$ (entry 12) produced a reversal of diastereomeric ratio.

Chiral amine **4b** (entry 2) also failed to provide good stereocontrol. Dimethylamine **4c** (entry 3) and cyclohexylamine **4e** (entry 5) followed the same trend, providing synthetically useful yields and modest stereocontrol. Only *p*-anisidine **4d** (entry 4) failed to give rise to the aza-Michael addition, owing to its lower nucleophilicity with respect to aliphatic amines. The reactivity of β-amino-alcohols was investigated next (entries 6–10). As in the case of simple amines, (*S*)-phenylalaninol (**4f**, entry 6), (*S*)-valinol (**4g**, entry 8) and (*S*)-phenylglycinol (**4h**, entry 10) gave rise to high-yielding but scarcely stereoselective reactions. It is worth noting that these reactions

Table 1. The conjugate additions performed as in Scheme 3

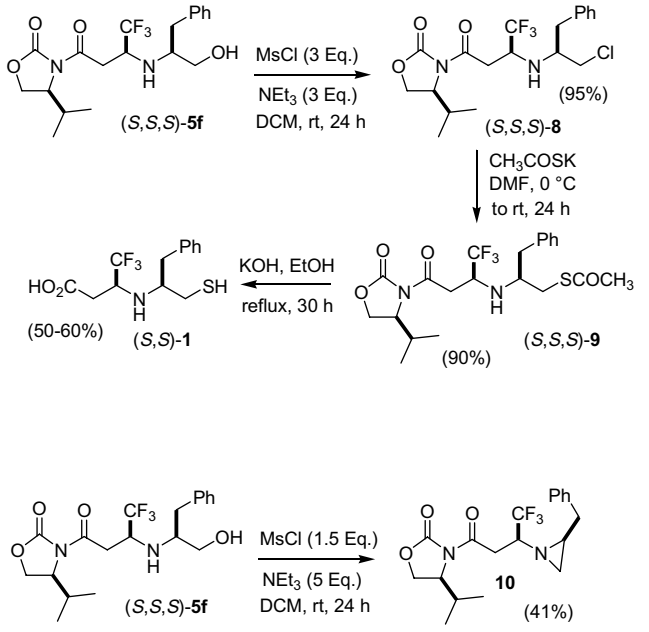
Entry	Amine	Conditions	Diast. ratio	Yield[a] (%)
1	**4a** (2 equiv)	Triethylamine (2 equiv), acetic acid (2 equiv), 2 h	1.3/1.0	90
2	**4b** (2 equiv)	Triethylamine (2 equiv), acetic acid (2 equiv), 3 h	1.7/1.0	67
3	**4c** (2 equiv)	Triethylamine (2 equiv), HCl (2 equiv), 3 h	1.0:1.0	80
4	**4d** (2 equiv)	Triethylamine (2 equiv), acetic acid (2 equiv), 100 h	—	—
5	**4e** (2 equiv)	Triethylamine (2 equiv), acetic acid (2 equiv), 3.5 h	1.7/1.0	76
6	(*S*)-**4f** (2 equiv)	48 h	1.5/1.0	93
7	(*R*)-**4f** (2 equiv)	48 h	1.1/1.0	65
8	(*S*)-**4g** (2 equiv)	24 h	1.3/1.0	72
9	(*R*)-**4g** (2 equiv)	24 h	1.0/1.0	70
10	(*S*)-**4h** (2 equiv)	24 h	1.4/1.0	95
11	**4a** (2 equiv)	Triethylamine (2 equiv), (*S*)-phenylpropionic acid (2 equiv), 3 h	1.2/1.00	>98[c]
12	**4a** (1 equiv)	$MgClO_4$ (1 equiv), 2 h[e]	1.0/1.6	>98[c]
13[b]	**4a** (1 equiv)	$BF_3{\cdot}OEt_2$ (1 equiv), 24 h[d]	—	—
14	**4a** (1 equiv)	$TiCl_4$ (1 equiv), 4 h[b]	—	—
15	**4a** (1 equiv)	Me_2AlCl (1 equiv), 4 h[b]	1.0/1.0	Not determined[f]

[a] Isolated yields.
[b] The reaction was conducted at −78 °C.
[c] Measured by NMR.
[d] The reaction was conducted at −78 °C to rt.
[e] The reaction was conducted at 0 °C.
[f] The products were obtained in a mixture with about 20% of the starting material.

could be performed without acid catalysis, as a likely consequence of the lower nucleophilicity of β-amino-alcohols with respect to amines **4a**–**e**.[10] In order to understand whether a match/mismatch recognition with chiral acceptor **3** could be involved, the reactions of (*R*)-enantiomers of **4f**,**g** were also explored, but these reactions afforded a nearly perfect 1:1 ratio of the corresponding diastereomeric products.

In order to see whether the low diastereocontrol of these aza-Michael reactions could be due to interconversion of the epimeric products **5**, one of the pure diastereomers of **5g** (obtained by silica gel chromatography) was submitted to the exact reaction conditions in the presence of **4g** for 30 h at rt. No epimerization was observed (NMR control), thus confirming that the aza-Michael reactions of **3** with amines and amino-alcohols **4** occur under kinetic control.

The diastereomeric adducts **5f** were chosen as starting materials for the synthesis of the trifluoroethylamine mimic of retrothiorphan (Scheme 3). In order to transform the hydroxyl into thiol function, we found that treatment of diastereomerically pure (*S*,*S*,*S*)-**5f** with MsCl (3 equiv) and triethylamine (3 equiv) afforded in nearly quantitative yields the chloride (*S*,*S*,*S*)-**8**.[11] We could not isolate the intermediate mesylate that undergoes fast S_N2 reaction with the chloride counterion to give **8**. However, we found that increasing the ratio NEt_3/MsCl to 10:3, the main product became aziridine **10** that was isolated in rather modest yields. Treatment of (*S*,*S*,*S*)-**8** with AcSK produced the desired thiol acetate (*S*,*S*,*S*)-**9** in excellent yields. Unfortunately, a number of methods to achieve exocyclic oxazolidin-2-one cleavage (LiOOH, HCl, etc.) from **9** were unsuccessfully tried, generally resulting in intractable mixtures of compounds arising from oxidation of the thiol function, or other side reactions. Eventually, we found that treatment of (*S*,*S*,*S*)-**9** with KOH in degassed EtOH at reflux was suitable for the preparation of the target diastereopure retrothiorphan (*S*,*S*)-**1** in moderate yields.[12] However, the reaction suffered from somewhat low reproducibility, occasionally providing large amounts of the undesired disulfide dimer of **1** together with other unidentified by-products. Indeed, the reaction outcome was apparently strongly dependent on the degree of purity of solvents and reagents employed. We therefore decided to develop an alternative procedure, by-passing the critical oxazolidin-2-one cleavage. Due to the low stereodirecting effect of the oxazolidin-2-one function, we sorted out to drop this auxiliary and use commercial ethyl trifluorocrotonate as starting Michael acceptor (Scheme 4).

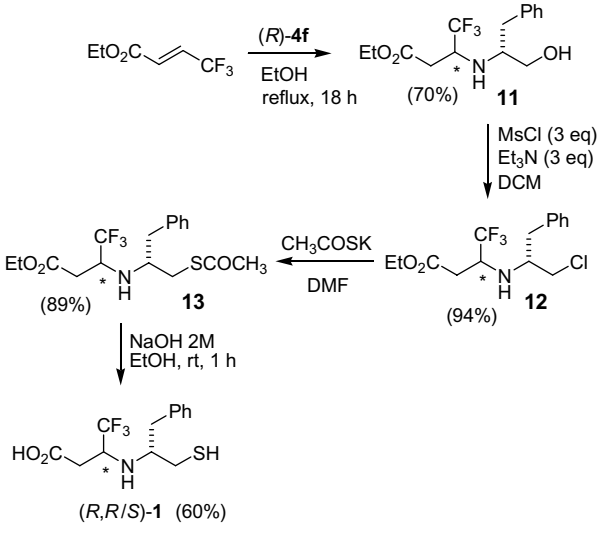


Scheme 3. Synthesis of stereochemically pure ψ[NHCH(CF_3)]-retro-thiorphan.

Addition of (*R*)-**4f** to ethyl trifluorocrotonate took place in good yields in EtOH at reflux, even though no stereocontrol was observed in this case too. Product **11** was obtained in an equimolar mixture of epimers at the CF_3-substituted carbon, which was very hardly separable by flash chromatography. Epimers **11** were therefore treated with MsCl according to the previously developed conditions, affording chlorides **12**, which were transformed into the epimeric thiolacetates **13**. The final cleavage of both terminal ester and thiolester functions was achieved by basic hydrolysis that provided the target retrothiorphan **1** having *R*-configuration at the benzylic stereocentre in satisfactory yields. In this case, the reaction was perfectly reproducible, although we were unable to separate the two epimers.[13]

The same reaction sequence portrayed in Scheme 4 was applied on enantiomer (*S*)-**4f**, thus leading to the epimeric retrothiorphan mimic (*S*,*R*/*S*)-**1**, obtained also in this case as an equimolar mixture of epimers at the CF_3-stereocentre.

All the ψ[NHCH(CF_3)]-retro-thiorphan diastereomers **1** were subjected to biological tests (fluorometric assay) to evaluate their inhibitory capacity toward Neutral



Scheme 4. Alternative route to ψ[NHCH(CF_3)]-retro-thiorphan (mixture of epimers).

Endopeptidase 24.11 (NEP). The assay was carried out by a method based on the procedure reported by Florentin et al.[14] All the ψ[NHCH(CF_3)]-retro-thiorphan diastereomers **1** showed IC_{50} values several orders of magnitude higher than thiorphan, with K_i values over 4 M (for reference compound: $IC_{50} = 5.06$ nM, $K_i = 2.53$ nM). Moreover, the comparison of the results obtained for the new ψ[NHCH(CF_3)] isosteres **1** with the data reported for (*R*) and (*S*)-retro-thiorphan ($K_i = 2.3$ nM and 210 nM, respectively)[15] confirmed the loss of the NEP inhibition capacity. This dramatic drop of inhibitory activity might be due to the fact that the retropeptidic carbonyl group of retro-thiorphan is known to be involved in critical interactions with the active site of NEP as hydrogen bond acceptor.[16] Therefore its replacement with the trifluoroethylamine function, which is a very weak hydrogen bond acceptor,[17] could be the underlying reason for the loss of potency. This observation suggests important considerations for a successful use of the trifluoroethylamine function as a peptide/retropeptide bond mimic (Fig. 2). (1) The trifluoromethyl group, contrarily to the carbonyl oxygen, is a weak hydrogen-bond acceptor.[17] The trifluoroethylamine function can be therefore an effective peptide bond replacement only if the carbonyl group of the original ligand's amide/peptide-bond is not involved in essential hydrogen-bonding with the receptor. (2) The NH of the trifluoroethylamine unit is a good hydrogen-bond donor, due to the strong electronwithdrawing effect exerted by the CF_3 group, and could be always considered a good mimic of a peptidic NH. (3) 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.[4a]

In summary, we have described the aza-Michael addition of amines and β-amino-alcohols to the chiral *N*-trifluorocrotonoyl-oxazolidin-2-one **3**. The reactions occur in good to excellent yields, but low stereocontrol. One of the aza-Michael adducts (**5f**) was used as the starting material for the synthesis of the stereopure ψ[NHCH(CF_3)] analogue **1** of retro-thiorphan. An alternative route to **1**, obtained in this case as an epimeric mixture at the CF_3-substituted stereocentre, has been developed as well from ethyl trifluorocrotonate. Unfortunately, all the diastereomers of **1** showed low inhibitory activity of NEP compared with the reference compounds thiorphan and retro-thiorphan.

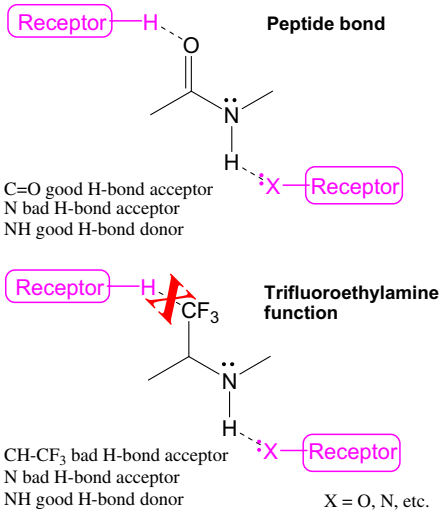


Figure 2. Comparison between the peptide (amide) bond and the trifluoroethylamine function.

Acknowledgments

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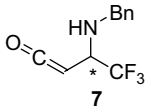
8. *Synthesis of* **5a**. TEA (2 equiv) and AcOH (2 equiv) were added at rt to a solution of **3** (1 equiv) in DCM (0.2 M solution). After 5 min **4a** was added at rt and the reaction monitored by TLC. The mixture was diluted with 1 N aqueous HCl solution, extracted with DCM, the collected organic layers dried (Na_2SO_4), filtered, concentrated under reduced pressure, and the crude purified by flash chromatography. **5a** (one diastereoisomer): ^1H (400 MHz, $CDCl_3$): δ 7.29 (m, 5H), 4.39 (m, 1H), 4.21 (m, 2H), 4.03 (d, $J = 13.2$ Hz, 1H), 3.85 (d, $J = 13.2$ Hz, 1H), 3.79 (m, 1H), 3.43 (dd, $J = 16.2$ and 9.4 Hz, 1H), 3.11 (dd, $J = 16.2$ and 4.1 Hz, 1H), 2.39 (m, 1H), 0.92 (d, $J = 7.1$ Hz, 3H), 0.84 (d, $J = 6.8$ Hz, 3H).

9. Another possible pathway to **6** could be the fragmentation into ketene **7** and the corresponding deacylated 2-oxazolidinone, followed by reaction with benzylamine.



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The protic acids successfully used in Table 1, entries 1–5,11 could therefore play the role of rapidly quenching the intermediate α-carbonyl carbanion formed by aza-Michael addition, thus suppressing the fragmentation via ketene **7**. The formation of ketenes by fragmentation of *N*-acyl-oxazolidinones has been observed previously: Bravo, P.; Fustero, S.; Guidetti, M.; Volonterio, A.; Zanda, M. *J. Org. Chem.* **1999**, *64*, 8731–8735 and references cited therein. Attempts to isolate these hypothetic ketene intermediates by adding external nucleophiles, such as dialkylamines, in the reaction mixture were unsuccessful.

10. β-Amino-alcohols are known to have a nucleophilic reactivity in Michael-type reactions which is intermediate between that of amines and α-amino-acids. See for example: Um, I.-H.; Lee, E.-J.; Seok, J.-A.; Kim, K.-H. *J. Org. Chem.* **2005**, *70*, 7530–7536. However, a referee suggested that the difference in reactivity observed between amines and β-amino-alcohols could lie in the fact that the latter can immediately protonate the carbanion formed after aza-Michael addition of the amino moiety to **3**. In our opinion, this hypothesis does not explain why α-amino-acid esters, which lack this proton, have the same reactivity of β-amino-alcohols, as shown in Ref. 3a,b.
11. *Synthesis of* **8**. Dry TEA (3 equiv) followed by freshly distilled MsCl (3 equiv) was added at rt under N_2 to a solution of (*S*,*S*,*S*)-**5f** (1 equiv) in dry DCM (0.1 M solution). After 24 h the mixture was diluted with water and extracted with DCM. The collected organic layers were dried (Na_2SO_4), filtered, concentrated under reduced pressure, and the crude was purified by flash chromatography. **8**: ^{1}H (400 MHz, $CDCl_3$): δ 7.33–7.18 (m, 5H), 4.41 (m, 1H), 4.23 (m, 2H), 3.86 (m, 1H), 3.49–3.32 (m, 4H), 3.04 (dd, $J = 16.6$ and 3.9 Hz, 1H), 2.85 (dd, $J = 13.5$ and 7.3 Hz, 1H), 2.74 (dd, $J = 13.5$ and 6.2 Hz, 1H), 2.40 (m, 1H), 1.61 (br s, 1H), 0.91 (d, $J = 6.9$ Hz, 3H), 0.86 (d, $J = 6.9$ Hz, 3H).
12. (a) The stereochemistry was assigned by X-ray diffraction of a single crystal of (*S*,*S*)-**1**. The crystallographic data will be reported in a forthcoming full letter. (b) *Synthesis of (S, S)*-**1**. Solid KOH (8 equiv) was added to a solution of (*S*,*S*,*S*)-**9** (1 equiv) in degassed abs. EtOH (0.1 M solution). The mixture was refluxed for 30 h., cooled to rt and the solvent evaporated. The crude was dissolved in a 1 N aqueous HCl solution and extracted several times with AcOEt. The collected organic layers were dried (Na_2SO_4), filtered, concentrated under reduced pressure, and the crude was purified by flash chromatography. (*S*,*S*)-**1**: ^{1}H (400 MHz, CD_3OD): δ 7.29–7.18 (m, 5H), 3.71 (m, 1H), 3.16 (m, 1H), 2.78 (m, 2H), 2.67 (dd, $J = 16.0$ and 4.2 Hz, 1H), 2.63 (dd, $J = 14.0$ and 4.7 Hz, 1H), 2.44 (dd, $J = 16.0$ and 9.4 Hz, 1H), 2.39 (dd, $J = 13.6$ and 4.7 Hz); ^{13}C (100 MHz, CD_3OD): δ 173.7, 139.9, 130.4, 129.5, 129.3, 127.9 (q, $J = 283.7$ Hz), 126.5, 60.2, 55.8 (q, $J = 28.4$ Hz), 40.7, 36.2, 28.4 ; $[\alpha]_D$ −23.3 (*c* 0.6, methanol).
13. Compounds **1** are indefinitely stable upon storage at 4 °C (neat). However, the stability is much lower when diastereomers **1** are stored in solution at room temperature, and definitely low when heated in the presence of bases, and in general at pH > 7. This precluded, in our hands, the possibility to isolate diastereopure **1** by crystallization with chiral bases.
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