Available online at www.sciencedirect.com
SCIENCE DIRECT®
Tetrahedron Letters 45 (2004) 1611–1615
Tetrahedron Letters

Synthesis and evaluation of stereopure α-trifluoromethyl-malic hydroxamates as inhibitors of matrix metalloproteinases

Monica Sani,[a,b] Dorina Belotti,[c] Raffaella Giavazzi,[c] Walter Panzeri,[b] Alessandro Volonterio[a,*] and Matteo Zanda[b,*]

[a]*Dipartimento di Chimica, Materiali ed Ingegneria Chimica "G.Natta" del Politecnico di Milano, via Mancinelli 7, I-20131 Milan, Italy*
[b]*CNR—Istituto di Chimica del Riconoscimento Molecolare, sezione "A. Quilico", via Mancinelli 7, I-20131 Milan, Italy*
[c]*Istituto di Ricerche Farmacologiche Mario Negri, Laboratori Negri, via Gavazzeni 11, I-24125 Bergamo, Italy*

Received 9 December 2003; revised 22 December 2003; accepted 23 December 2003

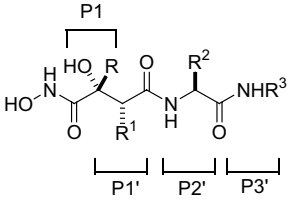
Abstract—The total synthesis of trifluoromethyl (Tfm) analogs of known nanomolar matrix metalloproteinases (MMPs) inhibitors has been performed. The synthetic protocol is based on a moderately stereoselective aldol reaction of trifluoropyruvate with an *N*-acyl-oxazolidin-2-thione for the construction of the core α-Tfm-malic unit. Both the diastereomeric forms of the target α-Tfm-malic hydroxamates showed micromolar inhibitory potency toward MMP-2 and 9, with a substantial drop with respect to the parent unfluorinated compounds.

Matrix metalloproteinases (MMPs) are zinc (II)-dependent proteolytic enzymes involved in the degradation of the extracellular matrix.[1] More than 25 human MMPs have been identified so far. Loss in the regulation of their activity can result in the pathological destruction of connective tissue, a process associated with a number of severe diseases, such as cancer and arthritis. The inhibition of various MMPs has been envisaged as a strategy for the therapeutic intervention against such pathologies. To date, however, a number of drawbacks have hampered the successful exploitation of MMPs as pharmacological targets. In particular, the toxicity demonstrated by many MMPs' inhibitors in clinical trials has been ascribed to nonspecific inhibition.

Incorporation of fluorine into organic molecules is an effective strategy for improving and modifying the biological activity.[2] In particular, the trifluoromethyl group is recognized in medicinal chemistry as a substituent of distinctive qualities. It is in fact at the same time highly hydrophobic, electron rich, sterically demanding, moreover it can provide high in vivo stability, and features a good mimicry with several naturally occurring residues such as methyl, isopropyl, phenyl, etc.[3]

Recently, Jacobson et al. described a new family of potent peptidomimetic hydroxamate inhibitors **A** (Fig. 1) of MMP-1, -3, and -9, bearing a quaternary α-methyl-alcoholic moiety at P1 position, and several different R^1 groups at P1′.[4] Interestingly, the other stereoisomers, including the epimers at the quaternary carbinol function, showed much lower activity, as the authors demonstrated that the hydroxamic binding function was moved away from the catalytic Zn^{2+} center.

Within the frame of a project aimed at studying the 'fluorine effect' in peptides and identifying selective



A R = CH_3; **1** R = CF_3

Figure 1. The DuPont Merck's MMP inhibitors (**A**) and their CF_3-analogs (**1**).

Keywords: Fluorine; Peptidomimetics; Matrix metalloproteinases.
* Corresponding authors. Tel.: +39-02-23993084; fax: +39-02-23993-080; e-mail addresses: alessandro.volonterio@polimi.it; matteo.zanda@polimi.it

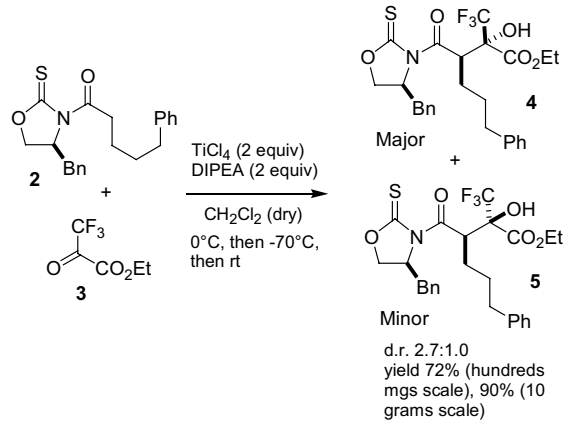
0040-4039/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2003.12.131

fluorinated inhibitors of aspartic proteinases and MMPs, we have recently described the synthesis, as well as the conformational and biological properties of several new types of fluorinated peptidomimetics.[5] We hypothesized that incorporation of fluoroalkyl substituents on the P1 quaternary position of the inhibitors **A** could be an effective strategy for optimizing and tuning their binding properties, thus increasing the selectivity toward the different MMPs. Now we describe the synthesis of the Tfm-analogs **1** (Fig. 1) of **A**, and the effect of the replacement of the α-CH_3 group with a CF_3 on the inhibition of MMP-9.

We decided to concentrate our efforts on the substrates **1** having $R^1 = (CH_2)_4$ Ph, whose analogs **A** were reported to be very active. The α-Tfm-malic unit of **1** was recently obtained by our group via titanium (IV) catalyzed aldol reaction of trifluoropyruvic esters with enantiopure *N*-acyl oxazolidin-2-ones.[6] Although this reaction was per se satisfactory (61–87% yields, dr up to 8:1 depending on the *N*-acyl group), the subsequent exocyclic cleavage of the oxazolidin-2-one auxiliary could not be performed, despite intensive efforts. We therefore decided to exploit the potential of oxazolidin-2-thiones,[7] whose cleavage has been reported to occur much more smoothly.[8]

The $TiCl_4$ catalyzed reaction of the *N*-acyl-oxazolidin-2-thione **2** (Scheme 1) with ethyl trifluoropyruvate **3** afforded the two diastereomeric adducts **4** and **5**, out of four possible, in low diastereomeric ratio. It is worth noting that the reaction features a favorable scale-up effect, affording ca. 70% yield on a 100-mg scale, and 90% on a 10-g scale (the reaction was repeated many times on both scales). A number of alternative conditions were explored, but neither significant improvement nor switch of the diastereocontrol could be achieved.[9]

Cleavage of the oxazolidin-2-thione was found to be considerably more challenging than expected. In fact, under the standard conditions reported in the literature (BnOH, cat. DMAP, DCM, rt) the reaction on **4** was very slow,[8] affording modest conversion to the corresponding Bn-ester **6** (60%) after one week at reflux, with



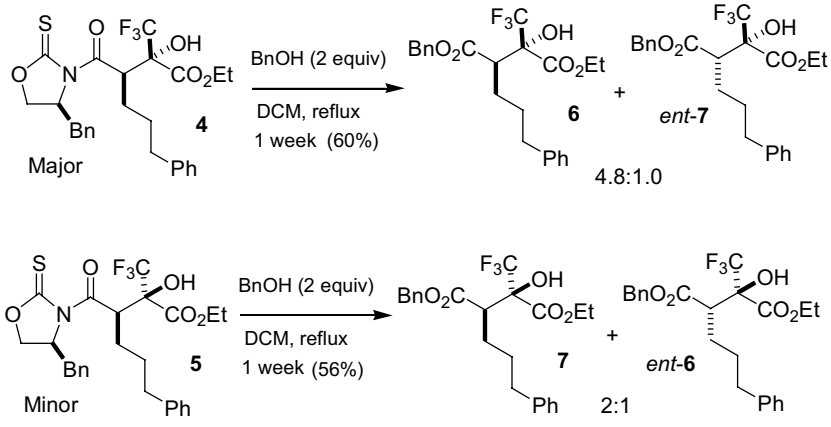
Scheme 1. The aldol reaction to form the α-Tfm-malic framework.

partial (17%) α-epimerization to give *ent*-**7**. Even less effectively, the same reaction performed on **5** gave 56% yield of the diastereomeric Bn-ester **7**, containing 33% of the α-epimer *ent*-**6**. Although the unreacted starting materials **4** and **5** could be recovered unchanged in good yields, we felt that more efficient conditions were needed in order to complete the synthesis. Disappointingly, exploration of several different combinations of alcohols, solvents, and bases did not improve the situation (Scheme 2).

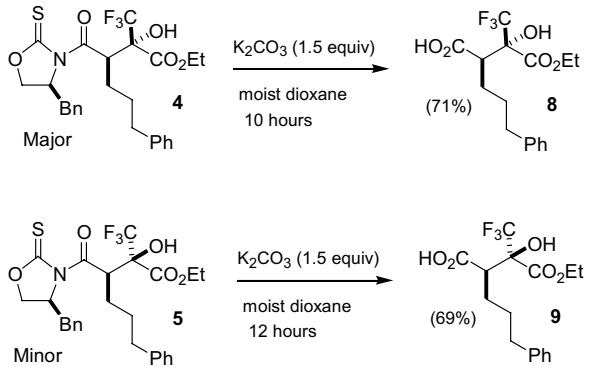
However, we were glad to find that the solid K_2CO_3 in moist dioxane (rt, 10–12 h), was able to produce directly the key carboxylic acid intermediates **8** and **9** (Scheme 3), from **4** and **5**, respectively, in satisfactory yields and with very low α-epimerization (2% for **8**, 9% for **9**).[10]

Coupling of the acid **8** with α-amino acid amides **10a–c** was achieved in good yields with the HOAt/HATU system (Scheme 4).[11] The resulting peptidomimetic esters **11a–c** were submitted to saponification, affording the acids **12a–c** in high yields.

The subsequent coupling of **12a–c** with O-Bn hydroxylamine proved to be extremely challenging, owing to the low reactivity and high steric hindrance of the carboxylic



Scheme 2. Attempted oxazolidin-2-thione cleavage with BnOH.



Scheme 3. Cleavage of the oxazolidin-2-thione auxiliary with K_2CO_3 in moist dioxane.

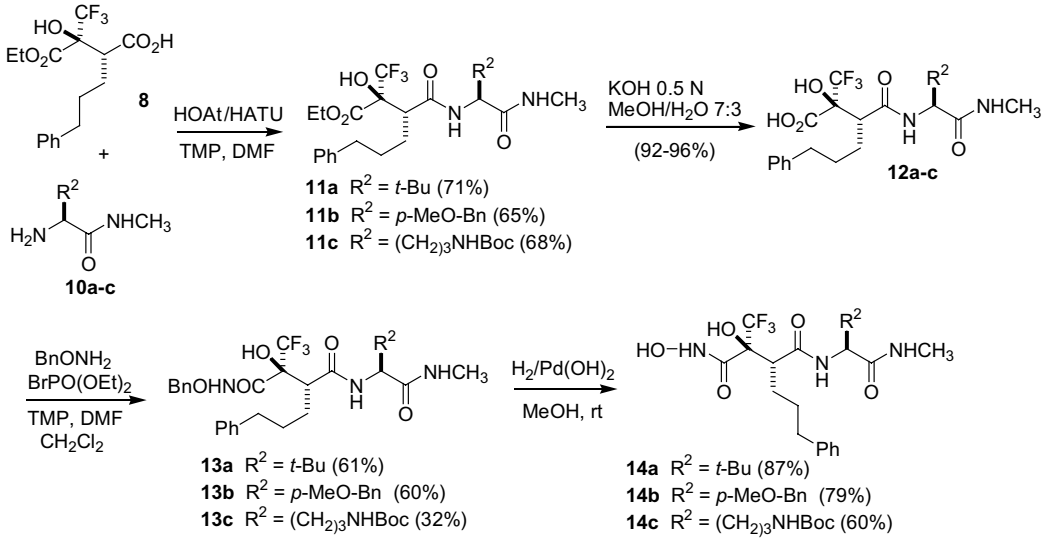
group bound the quaternary α-Tfm carbinolic center. A number of 'conventional' coupling agents for peptides[12] were tested (among them DCC/DMAP, EDC/HOBt, DIC/HOBt, HATU/HOAt, PyBroP/DIPEA) but no trace of the target O-Bn hydroxamates **13a–c** could be obtained. Finally, we found that freshly prepared $BrPO(OEt)_2$ was able to promote the coupling in reasonable yields (32–61%).[13] With **13a–c** in hand we addressed the final O-Bn cleavage by hydrogenolysis, that provided the hydroxamates **14a–c** in good yields.

Since **14a–c** are the 'wrong' diastereomers with respect to **A**, we deemed necessary to synthesize at least one analog having the correct stereochemistry, in order to have a complete set of biological data on the effect of the introduction of the Tfm group. However, a tailored synthetic protocol had to be developed *ex-novo*, because the minor diastereomer **9** (Scheme 5) featured a dramatically different reactivity in the key steps of the synthesis. First of all, we noticed that the coupling of **9** and **10a** with HATU/HOAt gave rise to relevant amounts of the β-lactone **15**, which had to be processed separately, besides the expected coupling product **16**. Thus, we decided to prepare first the intermediate **15** (72%), which could be purified by short flash chromatography (FC). The latter was reacted with free **10a**, affording the desired molecule **16** in high yields.[14]

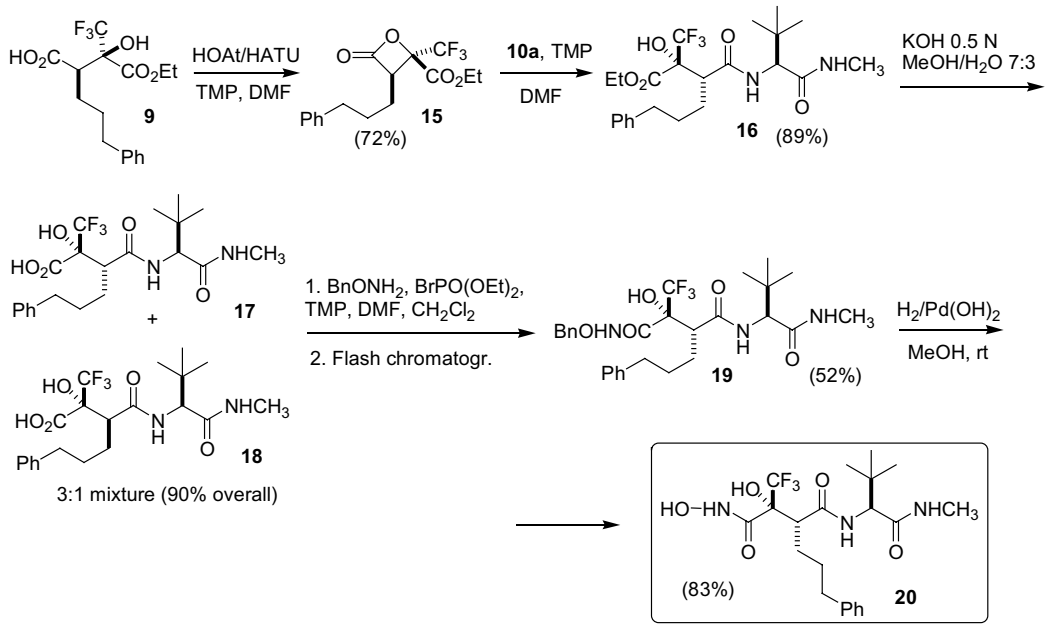
Saponification of the ester **16** occurred effectively, but disappointingly a partial epimerization of the $[Ph(CH_2)_3]$ stereocenter occurred, affording a 3:1 mixture of diastereomers **17** and **18** under optimized conditions. Since their chromatographic separation proved to be difficult, **17** and **18** were subjected together to coupling with $BnONH_2$ under the previously optimized conditions. The resulting diastereomeric O-Bn hydroxamates could be separated by FC, affording pure **19** (52%), that was hydrogenated to the target free hydroxamate **20** in 83% yield.

The hydroxamates **14a–c** and **20** where tested for their ability in inhibiting MMP-2 and MMP-9 activity using zymographic analysis. The four hydroxamates inhibited in a dose-dependent manner the gelatinolytic bands at 92 and 72 kDa, corresponding, respectively, to pro-MMP-9 and pro-MMP-2 released in the conditioned medium by human melanoma cells WM983A. The IC_{50} values (μM) portrayed in Table 1 show that diastereomers **14a–c** displayed low inhibitory activity, in line with the parent CH_3 compounds. Disappointingly, **20** showed a much lower activity than the exact CH_3 analog **A**, that was reported to be a low nanomolar inhibitor of MMP-9 (IC_{50} <1 nM toward MMP-9).[4a]

It is also worth noting that **14a** and **20** showed little selectivity, whereas **14b** and **14c** showed a fairly better affinity for MMP-9, in comparison with MMP-2. A possible explanation for the drop of activity upon replacement of the quaternary methyl with a CF_3, is that **20**, biased by the bulky CF_3 group, is unable to assume the crucial binding conformation of the CH_3 analog **A**. Alternatively, one can hypothesize that the bulky and highly electron-rich CF_3 group is unable to fit the S1 pocket of the hitherto tested MMPs.



Scheme 4. Synthesis of the peptidomimetics **14** from the major diastereomer **8**.



Scheme 5. Synthesis of the target peptidomimetic **20** from the minor diastereomer **9**.

Table 1. IC_{50} (μM) of the target Tfm-hydroxamates

Compound	MMP-2	MMP-9
14a	156	121
14b	407	84
14c	722	23
20	23	15

The preparation of other CF_3 peptidomimetics **1** is currently underway in order to have a more complete picture of the effect of the CF_3 introduction in this class of peptidomimetics. Computational studies are also in progress in order to understand the reasons for the unfavorable effect of the CF_3 group.[15]

Acknowledgements

We thank the European Commission (IHP Network grant "FLUOR MMPI" HPRN-CT-2002-00181), MIUR (Cofin 2002, Project "Peptidi Sintetici Bioattivi"), Politecnico di Milano, and CNR for economic support.

References and notes

1. (a) Coussens, L. M.; Fingleton, B.; Matrisian, L. M. *Science* **2002**, *295*, 2387–2392; (b) Whittaker, M.; Floyd, C. D.; Brown, P.; Gearing, A. J. H. *Chem. Rev.* **1999**, *99*, 2735–2776; (c) Bode, W.; Huber, R. *Biochim. Biophys. Acta.* **2000**, *1477*, 241–252; (d) Giavazzi, R.; Taraboletti, G. *Crit. Rev. Oncol. Hematol.* **2001**, *37*, 53–60.
2. Ojima, I.; McCarthy, J. R.; Welch, J. T. *Biomedical Frontiers of Fluorine Chemistry*; ACS Books, American Chemical Society: Washington, DC, 1996.
3. Banks, R. E.; Tatlow, J. C.; Smart, B. E. *Organofluorine Chemistry: Principles and Commercial Applications*; Plenum Press: New York, 1994.
4. (a) Jacobson, I. C.; Reddy, P. G.; Wasserman, Z. R.; Hardman, K. D.; Covington, M. B.; Arner, E. C.; Copeland, R. A.; Decicco, C. P.; Magolda, R. L. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 837–842; (b) Jacobson, I. C.; Reddy, G. P. *Tetrahedron Lett.* **1996**, *37*, 8263–8266.
5. See for example: (a) Molteni, M.; Volonterio, A.; Zanda, M. *Org. Lett.* **2003**, *5*, 3887–3890; (b) Volonterio, A.; Bellosta, S.; Bravin, F.; Bellucci, M. C.; Bruché, L.; Colombo, G.; Malpezzi, L.; Mazzini, S.; Meille, S. V.; Meli, M.; Ramírez de Arellano, C.; Zanda, M. *Chem. Eur. J.* **2003**, *9*, 4510–4522; (c) Sani, M.; Bruché, L.; Chiva, G.; Fustero, S.; Piera, J.; Volonterio, A.; Zanda, M. *Angew. Chem. Int. Ed.* **2003**, *42*, 2060–2063.
6. Zucca, C.; Bravo, P.; Malpezzi, L.; Volonterio, A.; Zanda, M. *J. Fluorine Chem.* **2002**, *114/2*, 215–223.
7. (a) Crimmins, M. T.; King, B. W.; Tabet, E. A.; Chaudhary, K. *J. Org. Chem.* **2001**, *66*, 894–902; (b) Crimmins, M. T.; McDougall, P. *J. Org. Lett.* **2003**, *5*, 591–594, and references cited therein; (c) Palomo, C.; Oiarbide, M.; García, J. M. *Chem. Eur. J.* **2002**, *8*, 37–44.
8. Su, D.-W.; Wang, Y.-C.; Yan, T.-H. *Tetrahedron Lett.* **1999**, *40*, 4197–4198.
9. Among the conditions explored: $TiCl_4$/(−)-sparteine (74% yield, **4**:**5** = 1.0/1.6); $Sn(OTf)_2/NEt_3$ (no reaction); Bu_2BOTf/NEt_3 (no reaction); LDA (48% yield, **4**:**5** = 2.6/1.0).
10. The stereochemistry of the α-Tfm-malic derivatives described herein was unambiguously determined by X-ray diffraction of a crystalline dipeptide derivative of **8**, not shown in this paper. Full details will be reported in a forthcoming full paper.
11. Carpino, L. A.; El Faham, A. *J. Org. Chem.* **1995**, *60*, 3561–3564, and references cited therein.
12. Humphrey, J. M.; Chamberlin, A. R. *Chem. Rev.* **1997**, *97*, 2243–2266.
13. (a) Gorecka, A.; Leplawy, M.; Zabrocki, J.; Zwierzak, A. *Synthesis* **1978**, 474–476, Contrary to the statement of these authors, $BrPO(OEt)_2$ undergoes rapid decomposition even at 4 °C, therefore it must be used within 1–2 h from its preparation. Our results are in agreement with an

earlier report; (b) Goldwhite, H.; Saunders, B. C. *J. Chem. Soc.* **1955**, 3564.
14. For the addition of amine nucleophiles to β-lactones see: Nelson, S. G.; Spencer, K. L.; Cheung, W. S.; Mamie, S. *J. Tetrahedron* **2002**, *58*, 7081–7091.
15. Key to the abbreviations and acronyms used in this paper: DCM = dichloromethane; TMP = *sym*-collidine (2,4,6-trimethylpyridine); DCC = dicyclohexylcarbodiimide; DMAP = 4-(*N*,*N*-dimethylamino)pyridine; EDC = 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride; HOBt = 1-hydroxybenzotriazole; DIC = diisopropylcarbodiimide; HATU = O-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate; HOAt = 1-hydroxy-7-azabenzotriazole; PyBroP = Bromotripyrrolidinophosphonium hexafluorophosphate; DIPEA = diisopropylethylamine; DMF = *N*,*N*-dimethylformamide.