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Synthesis of α-trifluoromethyl-α-amino-β-sulfone hydroxamates: novel nanomolar inhibitors of matrix metalloproteinases

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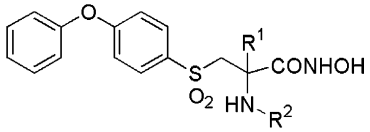
Abstract—The racemic α-trifluoromethyl-α-amino-β-sulfone hydroxamates **1** were synthesized by means of a nucleophilic addition of sulfur-stabilized carbanions to a *N*-Cbz imine of trifluoropyruvate (**4**). The free amino derivative **1a** was the most potent inhibitor of both MMP-3 (stromelysin-1) and MMP-9 (gelatinase-B), showing an $IC_{50} = 14$ nM and 1 nM, respectively, and excellent selectivity versus MMP-1 (>5000-fold difference in inhibitory capacity). The *N*-Me derivative **1b** was the most selective for MMP-3 with respect to MMP-9 (62-fold difference).

Matrix metalloproteinases (MMPs) are a family of zinc metalloendopeptidases secreted by cells, which are responsible for much of the turnover of matrix components.[1]

MMPs have been reported to play a key-role, in combination with their natural tissue inhibitors (TIMPs), in many serious diseases, such as heart failure and cancer. Progression and growth of both pathologies, mainly in the early stages, is favoured by the proteolytic activity of several MMPs, such as stromelysin-1 (MMP-3) and gelatinase-B (MMP-9). For these reasons, inhibition of MMPs is actively studied as a promising therapeutic target for heart failure and cancer therapy.[2]

Recently, Becker et al. described a family of α-alkyl-α-amino-β-sulfone hydroxamates **A** (Fig. 1) as potent inhibitors of MMP-2, MMP-9 and MMP-13, while exhibiting limited inhibition of MMP-1, an enzyme thought to be responsible of the musculoskeletal side effect observed clinically with the broad-spectrum MMP inhibitor marimastat.[3]



R^1 = H, CH_3, Ph (**A**)
R^1 = CF_3 (**1**, this work)

Figure 1.

Whereas a large number of different alkyl and alkylaryl residues were well tolerated as nitrogen substituents R^2, only inhibitors bearing $R^1 = H$, CH_3 or Ph were described.

Within the frame of a research project aimed at a better understanding and rationalization of the 'fluorine-effect' in peptidomimetic structures, particularly those displaying activity as protease inhibitors,[4] we decided to investigate the effect of a trifluoromethyl (Tfm) group, positioned as R^1 substituent of structures **A**, on the inhibition of MMPs activity. It is in fact widely recognized

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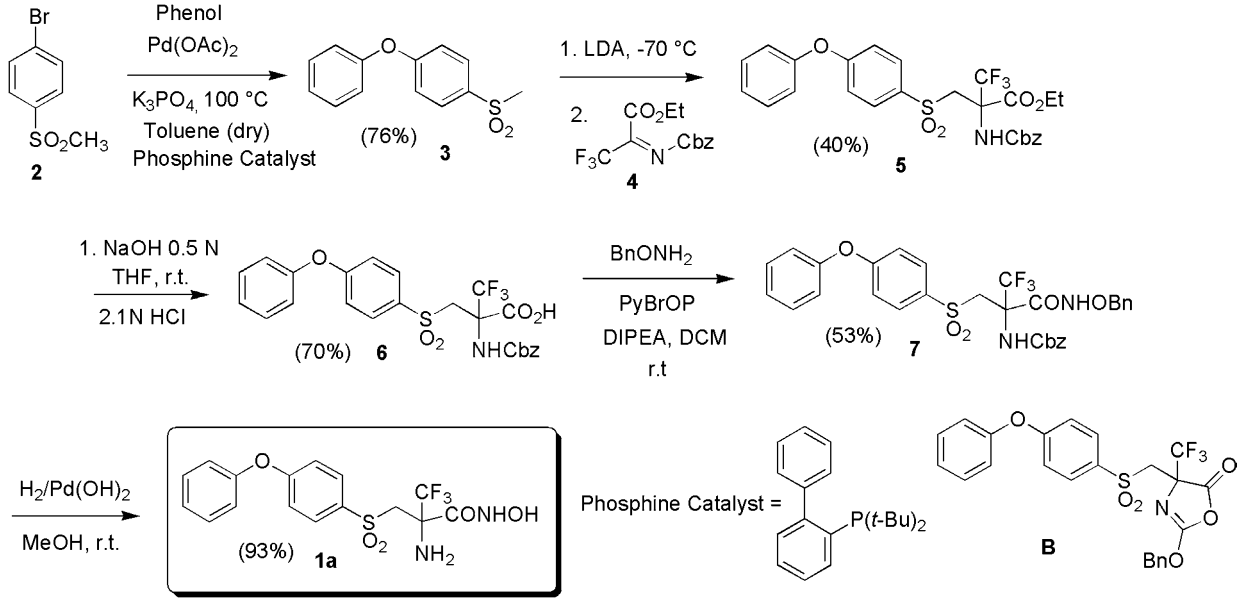


Scheme 1.

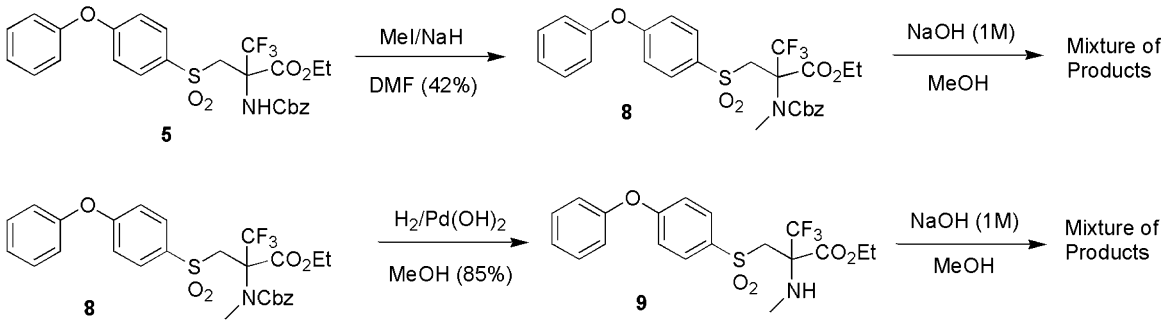
in medicinal chemistry that the Tfm group is a substituent of distinctive qualities. It is at the same time highly hydrophobic and sterically demanding.[5] Moreover, its high electron density could provide additional interactions within the MMPs active sites, possibly including hydrogen bonding.[6] In addition, the Tfm has been used to replace both methyl and phenyl groups.[5]

We first undertook the synthesis of the hydroxamic acid **1a**, having a free quaternary amino group (Scheme 1). The intermediate sulfone **3** was synthesized by Pd-catalyzed reaction of phenol with the *p*-Br derivative **2**.[7] Lithiation of **3**, followed by nucleophilic addition to the *N*-Cbz imine of trifluoropyruvate **4**[8] afforded the α-Tfm α-amino acid derivative **5** in fair yields.[9] Saponification of the ester function delivered the carboxylic acid **6**, that was subjected to condensation with *O*-Bn-hydroxylamine affording the hydroxamate **7** (oxazolin-5-one **B** was formed as co-product, in ca. 20% yield, in the condensation), which was hydrogenolyzed to the target molecule **1a**.[10]

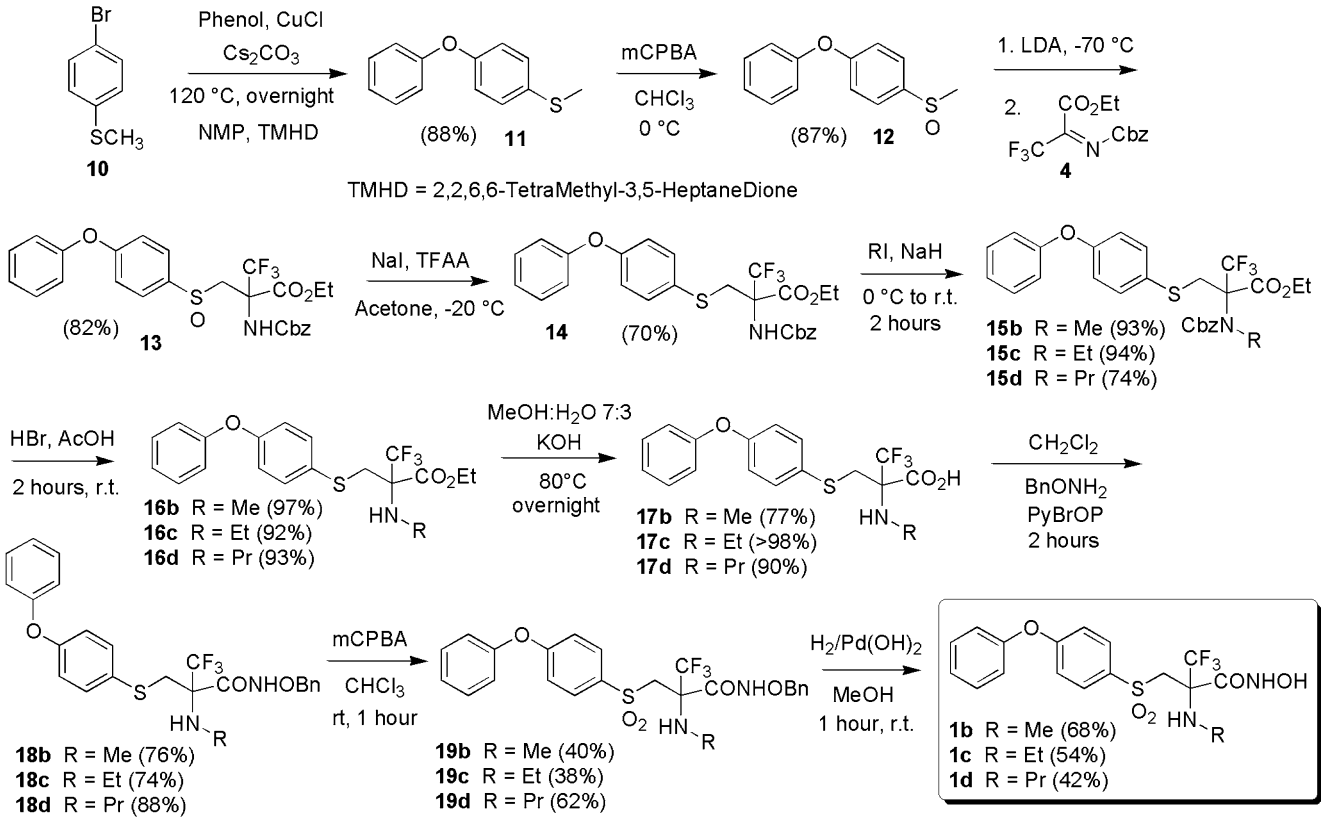
With **1a** in hand, we next addressed the synthesis of its *N*-alkyl derivatives. The challenging *N*-methylation of the α-Tfm-substituted compound **5** was achieved in acceptable yields (Scheme 2).[11] The resulting *N*-methyl-α-amino ester **8** was submitted to saponification, but the reaction was surprisingly slow and after three days a complex mixture of products was formed. Different basic conditions were attempted (such as LiOH and KOH), but no significant improvement could be achieved. We thus decided to hydrogenolyze first the Cbz group of **8**, which produced the expected secondary amine **9**. Unfortunately, also in this case we were unable to perform the final saponification step.

We therefore decided to re-investigate the synthetic protocol on the sulfanyl analogues of **8**, which were synthesized as portrayed in Scheme 3. To this end, the sulfanyl diaryl ether **11** was prepared from phenol and **10** according to a Ullmann-type reaction,[12] then oxidized to the sulfoxide **12**. Lithiation and Mannich-type reaction with **4** afforded a nearly equimolar mixture of sulfoxide diastereomers **13**, which were deoxygenated to the racemic sulfide **14** according to the Drabowicz and Oae protocol.[13]

N-Alkylation occurred in good to excellent yields affording the corresponding sulfides **15b–d**. Due to the presence of the sulfide function, which could interfere with



Scheme 2.



Scheme 3.

the hydrogenolysis, the Cbz was cleaved with HBr,[14] affording the secondary amines **16b–d** in nearly quantitative yields. To our satisfaction, the 'difficult' ester saponification step could be performed smoothly, affording the carboxylic acids **17b–d** in good to excellent yields.[15] Coupling of **17b–d** with *O*-Bn-hydroxylamine occurred smoothly affording the sulfanyl hydroxamates **18b–d**, which were oxidized to sulfones **19b–d**.[16] The target hydroxamic acids **1b–d** were obtained in fair yields by hydrogenolysis with the Pearlman catalyst.

With racemic **1a–d** in hand, we next addressed the inhibition tests on the catalytic domains of MMP-1, MMP-3 and MMP-9.[17]

The primary α-amino hydroxamate **1a** is the most potent compound (Table 1), but it is worth noting that all of them are nanomolar inhibitors of MMP-3 and MMP-9, with the exception of the *N*-Me derivative **1b** which showed good selectivity for MMP-3 (ca. 62-fold selective vs MMP-9). Even more importantly, **1a** showed excellent selectivity versus MMP-1 (>5000-fold). We tested the effect of the primary α-amino hydroxamate **1a** also on the gelatinolytic activity of full length MMP-9 secreted by macrophages in culture.[18] Interestingly, even in these different experimental conditions, the compound showed an inhibitory activity. These results show that a Tfm group can be successfully used as a substituent in protease inhibitors,[19] and is very well tolerated by the enzymes. On the other hand, previous results from our group showed that replacement of an α-methyl by Tfm group in another structural class of hydroxamate inhibitors of MMPs was responsible for a dramatic loss of inhibitory potency.[20] It was also shown that the final outcome of Tfm incorporation is strongly dependent on the whole structure of the inhibitor.[21]

Table 1. Effect of the compounds on different MMPs' proteolytic activity

Compound	IC_{50}/MMP-3 (nM)	IC_{50}/MMP-9 (nM)	IC_{50}/MMP-1 (nM)
1a	14	1	>5000
1b	32	1982	n.a.
1c	28	63	n.a.
1d	53	59	n.a.

n.a. = not available.

We are currently investigating the synthesis and the inhibitory activity of the single enantiomers of **1** on a wider range of MMPs, in order to have a more complete picture of the effect of fluorine in terms of potency and selectivity in this particular class of protease inhibitors. Attempts to obtain X-ray data on the co-crystals of **1** with MMP catalytic domains are also in progress, in order to have more detailed structural information about the role of the Tfm group in the binding process.

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9. (a) Several unidentified by-products formed, according to TLC monitoring.; (b) Synthesis of **5**: to a stirred solution of DIPEA (1.75 mL, 12.5 mmol) in dry THF (20 mL), cooled at −74 °C and under nitrogen, a 2.5 M solution of *n*-BuLi (5 mL, 12.5 mmol) was added and the solution was allowed to warm at 0 °C. The resulting solution was cooled at −78 °C, and a solution of sulfone **3** (2.4 g, 9.6 mmol) in dry THF (5 mL) was added. After 10 min, a solution of the crude imino derivative **4** (12.5 mmol) was added dropwise. After stirring for 2 h at −70 °C the reaction mixture was quenched with saturated aqueous NH_4Cl. The layers were separated and the aqueous phase was extracted with AcOEt. The collected organic phases were dried over anhydrous Na_2SO_4 and the solvent was removed in vacuo. The residue was purified by flash chromatography (*n*-hexane–AcOEt, 9:1), affording 2.12 g of **5** (40%); R_f: 0.43 (*n*-hexane–AcOEt, 8.2); FT-IR (film) ν_{max} 3403.0, 2986.7, 1732.6, 1488.6 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ: 7.81 (d, 2H, $J = 8.9$ Hz), 7.47–7.12 (m, 8H), 7.08–6.92 (m, 4H), 6.23 (s, 1H), 5.11 (d, 1H, $J = 14.1$ Hz), 4.95 (d, 1H, $J = 12.4$ Hz), 4.62 (d, 1H, $J = 12.4$ Hz), 4.45 (m, 2H), 3.96 (d, 1H, $J = 14.1$ Hz), 1.40 (t, 3H, $J = 7.3$ Hz); ^{13}C NMR (250 MHz, $CDCl_3$) δ: 164.3, 162.8, 154.5, 153.3, 135.2, 132.9, 130.8, 130.2, 128.6, 128.4, 127.9, 125.3, 120.4, 117.2, 67.1, 64.8, 62.7 (q, $J = 29.6$ Hz), 62.5, 52.5, 13.7, the CF_3 signal was obscured; ^{19}F NMR (235.3 MHz, $CDCl_3$) δ: −74.5 (s, 3F); MS (DIS EI 70 eV) *m*/*z* (%): 551 [M^+] (4), 233 (23), 108 (82), 91 (100).
10. Synthesis of **1a**: to a solution of **7** (100 mg, 0.16 mmol) in MeOH (3 mL), a catalytic amount of $Pd(OH)_2/C$ was added and the reaction mixture was kept vigorously stirred under hydrogen atmosphere at room temperature for 5 h. The catalyst was filtered over a Celite pad, and washed with MeOH. The solvent was removed in vacuo and the residue was purified by FC ($CHCl_3$–MeOH, 97:3), affording 60 mg of **1a** (93% yield); R_f: 0.17 ($CHCl_3$–MeOH, 95:5); FT-IR (film) ν_{max} 3011.3, 1688.8, 1584.3 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ: 10.21–9.38 (br s, 1H), 7.90 (d, 2H, $J = 8.5$ Hz), 7.58–7.21 (m, 4H), 7.18–7.02 (m, 3H), 6.03 (s, 1H), 4.22 (d, 1H, $J = 14.8$ Hz), 4.10–3.80 (br s, 2H), 3.48 (d, 1H, $J = 14.8$ Hz); ^{13}C NMR (250 MHz, $CDCl_3$) δ: 162.9, 154.5, 132.8, 130.7, 130.5, 125.1, 123.3 (q, $J = 280.4$ Hz), 62.7 (q, $J = 29.2$ Hz), 57.8; ^{19}F NMR (235.3 MHz, $CDCl_3$) δ: −74.4 (s, 3F); MS (DIS EI 70 eV) *m*/*z* (%): 405 [$M+H^+$] (22), 233 (100).
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15. The striking difference of reactivity in the saponification reaction between sulfone **9** and sulfides **16** could be due to the difference of acidity of the α-sulfonyl and the α-sulfanyl protons.
16. These oxidations performed by *m*-CPBA at rt occurred in modest to fair yields, as a likely consequence of side reactions involving the hydroxamate moiety and/or the electron-rich aryl groups. In fact, several unidentified by-products were detected by TLC monitoring.
17. Full length MMP-1 was purchased from Biomol, and the activity of **1a** measured by collagen gel zymography as previously described (see Ref. 18). The catalytic domains of MMP-3 and MMP-9 enzymes were produced in *E. coli*, transfected with cDNAs corresponding to the respective human sequences. Proteins were purified by affinity chromatography and the inhibitory potencies of racemic **1** and single enantiomers were assayed with synthetic, general MMP fluorescent substrate (Mca-PLGLDpaAR, Tebu-bio) using a FL600 Avantec fluorimeter. For MMP-3 see: (a) Ye, Q. Z.; Johnson, L. L.; Hupe, D. J.; Baragi, V. *Biochemistry* **1992**, *31*, 11231–11235; For MMP-9 see: (b) Ye, Q. Z.; Johnson, L. L.; Yu, A. E.; Hupe, D. *Biochemistry* **1995**, *34*, 4702–4708.
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