



# Solution/solid-phase synthesis of partially modified retro- $\psi$ [NHCH(CF<sub>3</sub>)]-peptidyl hydroxamates

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**Abstract**—The synthesis of a novel family of partially-modified (PM) retropeptidyl hydroxamates incorporating a [CH(CF<sub>3</sub>)CH<sub>2</sub>CO] unit as a surrogate of the conventional malonyl group, has been accomplished both in solution and in solid-phase. The key step is the Michael-type *N*-addition of free or polymer bound  $\alpha$ -amino hydroxamates to 3-(*E*-enoyl)-1,3-oxazolidin-2-ones, which takes place very effectively, although with low stereocontrol. A number of tri- and tetra-peptidyl hydroxamates were obtained either in diastereomerically pure form (by solution-phase synthesis, after chromatographic purification), or as mixtures of two epimers in very good chemical purity (by solid-phase, after release from the resin), demonstrating that this method is suitable for preparing combinatorial libraries of PM retro- $\psi$  [NHCH(CF<sub>3</sub>)]-peptidyl hydroxamates for screening as metalloprotease inhibitors. © 2001 Elsevier Science Ltd. All rights reserved.

## 1. Introduction

The search for high-affinity, selective and relatively non-toxic inhibitors of metalloproteases is a very important issue in the pharmaceutical field.<sup>1</sup> These proteolytic enzymes 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.<sup>2</sup> The field of synthetic metalloprotease inhibitors is dominated by compounds containing a terminal hydroxamate function, since the HONHCO- end group is very effective in coordinating the Zn<sup>2+</sup> cofactor of metalloproteases.<sup>3</sup> 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.<sup>4</sup> This paper describes the efficient solution and solid-phase synthesis of partially-modified (PM) retro- $\psi$  [NHCH(CF<sub>3</sub>)]-peptidyl hydroxamates, a novel class of hydroxamates incorporating a [CH(CF<sub>3</sub>)CH<sub>2</sub>CO] unit as a surrogate of the malonyl moiety featured in conventional PM retropeptides (Fig. 1).<sup>5</sup>

## 2. Results and discussion

### 2.1. Solution-phase synthesis

The backbone of PM retro- $\psi$  [NHCH(CF<sub>3</sub>)]-peptidyl hydroxamates was built through a Michael-type *N*-addition of amino acid-derived *O*-Bn hydroxamates **1** with the chiral 3-(*E*-enoyl)-1,3-oxazolidin-2-one **2** (Scheme 1).<sup>6</sup> Compounds **1** were prepared by 1-hydroxy-benzotriazole/diisopropylcarbodiimide (HOBt/DIC) promoted condensation of *N*-Boc  $\alpha$ -amino acids with *O*-Bn-hydroxylamine hydrochloride in DMF/triethylamine (TEA), followed by *N*-Boc-cleavage with trifluoroacetic

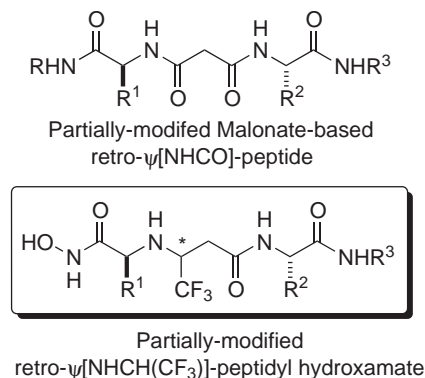
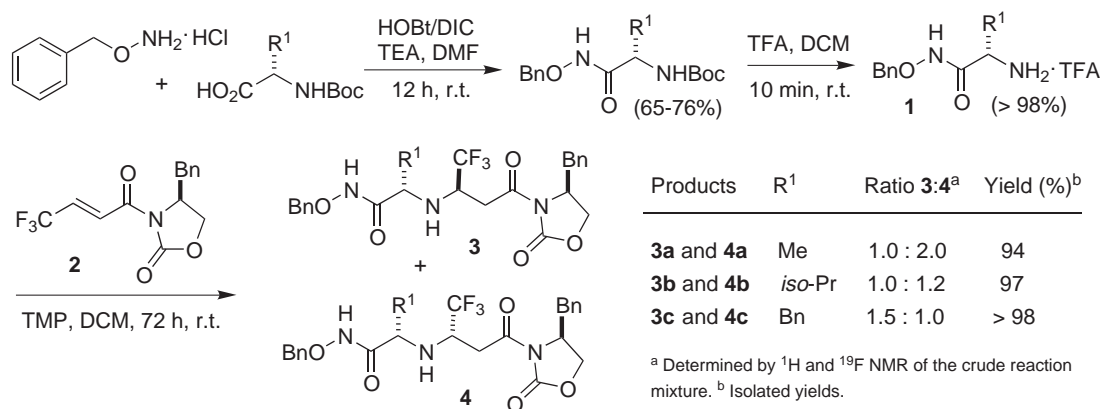


Figure 1.

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Scheme 1.

acid (TFA) in dichloromethane (DCM). The diastereomeric adducts **3** and **4a–c** were formed by conjugate addition of **1** to **2** (2 equiv.) in DCM/*sym*-collidine (TMP) (2 equiv.) for 72 h at rt. These reactions were very clean and high yielding, although low diastereocontrol was achieved in all cases.<sup>7</sup> However, in view of combinatorial applications the low stereocontrol is not necessarily a drawback, because both epimers at the trifluoromethyl (Tfm) stereocentre can be produced. Diastereomerically pure **3** and **4a–c** were isolated by flash chromatography (FC).

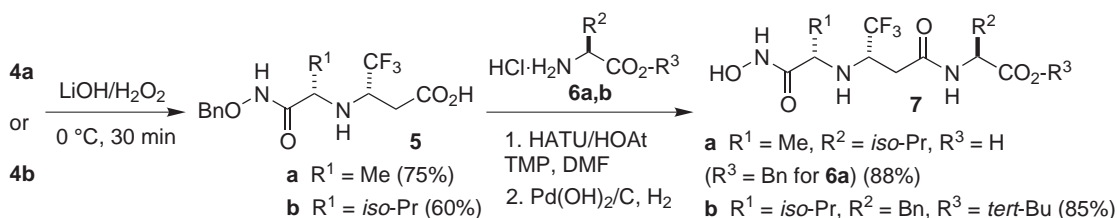
Exocyclic cleavage of the oxazolidin-2-one was performed by treatment of **4a** and **4b** with in situ generated lithium hydroperoxide (Scheme 2),<sup>8</sup> which delivered the expected acids **5** without affecting the hydroxamate moiety. The target PM retro-tripeptidyl hydroxamates **7a** and **7b** were obtained via HATU/HOAt<sup>9</sup> promoted coupling with L-Val-OBn (**6a**) and L-Phe-O-*tert*-Bu (**6b**), respectively, followed by catalytic hydrogenolysis of the terminal OBn groups.

## 2.2. Solid-phase synthesis

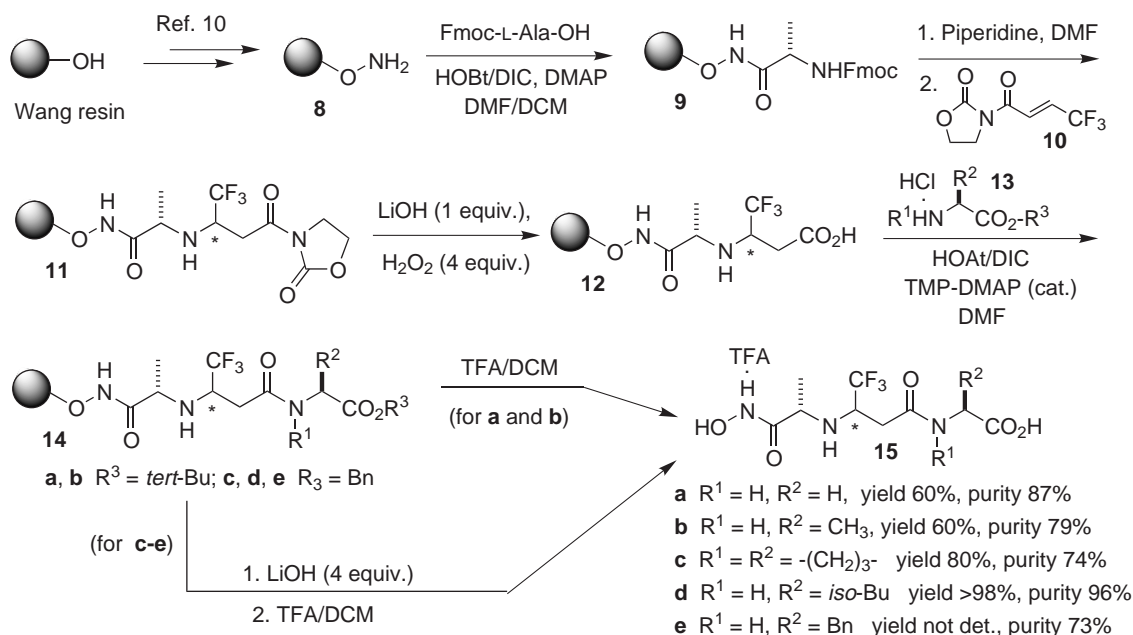
This method was also adapted to the solid-phase, with the view of preparing combinatorial libraries of PM retro-ψ[NHCH(CF<sub>3</sub>)]-peptidyl hydroxamates for screening as metalloprotease inhibitors. First, we addressed the preparation of retro-tripeptidyl hydroxamates **15**, having a CO<sub>2</sub>H terminus (Scheme 3). The hydroxylamine resin **8** was prepared in two steps from commercial Wang resin, according to the method of Floyd,<sup>10</sup> and coupled to an excess of L-Fmoc-Ala to give the protected alanine polymer **9**, from which the Fmoc group was cleaved with 20% piperidine in DMF.

The resulting resin-bound α-amino hydroxamate was submitted to 1,4-conjugate addition with the achiral oxazolidin-2-one **10** (3 equiv. in DCM, 3 days, rt). The FT-IR spectrum of the resulting Tfm-resin **11** exhibited a strong O(CO)N band at 1785 cm<sup>-1</sup>, totally absent in the precursor **9**. Treatment of **11** with lithium hydroxide (1 equiv. based on the theoretical loading) and hydrogen peroxide (4 equiv.) in THF/H<sub>2</sub>O (0°C, 2 h) cleaved the oxazolidin-2-one with excellent chemoselectivity. As a result, the FT-IR of the CO<sub>2</sub>H resin **12** showed disappearance of the O(CO)N band at 1785 cm<sup>-1</sup>, and transformation of the amide band (1700 cm<sup>-1</sup>) into an intense band at 1620 cm<sup>-1</sup>, which could be assigned to the carboxyl residue. Coupling of **12** to α-amino esters **13** (DIC/HOAt, TMP/DMAP<sup>11</sup>) afforded the tripeptidyl resins **14**, from which the hydroxamates **15** were released in good yields and purity upon treatment with TFA/DCM (1 h, rt).<sup>12</sup> As expected, <sup>1</sup>H and <sup>19</sup>F NMR spectroscopy showed that **15a–e** were formed as nearly equimolar mixtures of epimers at the Tfm-substituted centre. For R<sup>3</sup> = *tert*-butyl, TFA treatment resulted in concomitant hydrolysis of the terminal ester function delivering the free carboxyl derivatives **15a,b**, which were found to be indefinitely stable upon storage at 4°C.

In contrast, the hydroxamates having a terminal benzyl ester function, obtained directly from **14c–e** by TFA release, were found to be rather unstable under the same storage conditions, producing complex mixtures of products within a few days. Thus, the ester functions of polymers **14c–e** were first hydrolysed with lithium hydroxide, then TFA treatment of the resins released the stable carboxyl-free hydroxamates **15c–e**, with very good overall yields and purities.



Scheme 2.



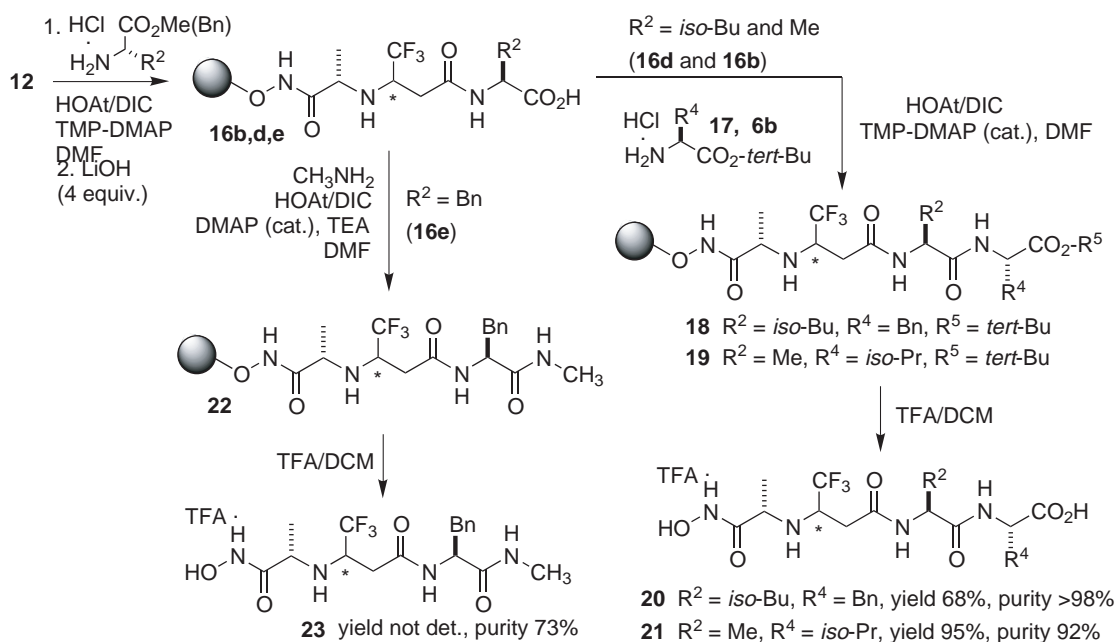
Scheme 3.

Next, in order to demonstrate the versatility of the method, we addressed the solid-phase preparation of tetrapeptidyl hydroxamates having a  $\text{CO}_2\text{H}$  terminus (**20** and **21**), and tripeptidyl hydroxamates having a methylamide terminus (**23**), which is often encountered in metalloprotease inhibitors (Scheme 4).<sup>1</sup> Tripeptidyl hydroxamate polymers **16**, having a  $\text{CO}_2\text{H}$  end-group, were obtained from **12** following the established protocol. Coupling of **16d** and **16b** to L-Phe-O-*tert*-Bu (**6b**) and L-Val-O-*tert*-Bu (**17**) (HOAt/DIC, TMP/DMAP), afforded the resin-bound tetrapeptidyl derivatives **18** and **19**, respectively. The free hydroxamates **20** and **21**

were released with TFA/DCM in excellent purity as mixtures of two epimers.

Finally, the retro-tripeptidyl hydroxamic amide **23** was prepared in good purity upon coupling of the polymer **16e** to methylamine (HOAt/DIC, TEA/DMPAP), followed by the usual release with TFA/DCM.

In summary, we have developed both a solution and a solid-phase protocol to prepare a novel structural family of fluorinated retro-peptidyl hydroxamates. Although in this work we did not perform a true



Scheme 4.

combinatorial approach to the title compounds, the examples shown provide good evidence that the method is general and could be applied for that purpose. Evaluation of some of the title compounds as matrix metallo-protease inhibitors is currently in progress, and will be reported in a full paper.

### Acknowledgements

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- (a) These Michael-type additions with  $\alpha$ -amino hydroxamates are remarkably less stereoselective than those involving the corresponding  $\alpha$ -amino esters (see Ref. 5a,b). For example, L-Val-OBn reacted with **2** providing an 86:14 ratio in favour of the **4b** analogue. The reasons for this rather surprising drop of stereoselectivity are presently unclear, but we have found that the steric hindrance of the CO<sub>2</sub>R has a minor influence on the stereocontrol of  $\alpha$ -amino ester additions. In fact, nearly the same degree of stereoselectivity was observed when R=benzyl or *tert*-butyl in the case of L-Phe-OR. This suggests that the low stereocontrol observed in this work could be due to the fact that  $\alpha$ -amino acid-derived esters and hydroxamates react with **2** through different conformations, with a weaker stereodirecting effect in the case of hydroxamates. (b) Excess of **2** could be recovered quantitatively by FC. (c) The absolute stereochemistry of hydroxamates **3** and **4**, and their derivatives, was unambiguously assigned by means of X-ray diffraction and chemical correlation with known  $\psi$ [NHCH(CF<sub>3</sub>)]-retropeptides (see Ref. 5a). The details will be published in a full paper.
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- Yields were calculated on the amount of final products released from the resins, referring to the loading of the starting Wang resin (1.3 mmol/g).