Solution/Solid-Phase Synthesis of Partially Modified Retro- and Retro-Inverso-ψ[NHCH(CF_3)]-Peptidyl Hydroxamates and Their Evaluation as MMP-9 Inhibitors

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The synthesis of a novel family of partially modified (PM) retro- and retro-inverso-peptidyl hydroxamates, each incorporating a [CH(CF_3)CH_2CO] unit as a surrogate for 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 α-amino hydroxamates to 3-[(*E*)-enoyl]-1,3-oxazolidin-2-ones, which takes place in high yields, although with low stereocontrol. This method is suitable for the preparation of combinatorial libraries of PM retro-ψ[NHCH(CF_3)]-peptidyl hydroxamates for screening as metalloprotease inhibitors. A number of tri- and tetrapeptidyl hydroxamates were indeed obtained either in diastereomerically pure form by solution-phase synthesis followed by chromatographic purification, or as mixtures of two epimers by solid-phase synthesis and release from the resin. X-ray diffraction of a Tfm-retropeptidyl hydroxamate showed an interesting turn-like conformation with an intramolecularly hydrogen-bonded nine-membered ring, and a nearly planar geometry of the NH group bound to the CH(CF_3) group. Three retro-peptidyl hydroxamates were submitted to bioassays, and displayed the capacity to reduce MMP-9 (Gelatinase B) gelatinolytic activity.

Introduction

The design and synthesis of selective, small molecule inhibitors of matrix metalloproteinases (MMPs) is currently an attractive target in the pharmaceutical field.[1] These proteolytic enzymes have been implicated in a number of inflammatory and degenerative diseases, such as arthritis, arteriosclerosis, stroke, and cancer, and so their inhibition constitutes a primary therapeutic target.[2] The field of synthetic metalloprotease inhibitors is dominated by compounds containing a terminal hydroxamate function, since the HONHCO endgroup is very effective in coordinating the Zn^{2+} cofactor of metalloproteases.[3] This explains the current interest in the development of novel synthetic routes and novel structural classes of hydroxamate peptidomimetics, possibly by solid-phase/combinatorial techniques, which provide ready access to libraries of compounds for fast simultaneous screening, from which the most potent inhibitors may be selected.[4] This paper describes in full detail the solution and solid-phase synthesis of partially modified (PM) retro-ψ[NHCH(CF_3)]-peptidyl hydroxamates, a novel class of hydroxamates incorporating a [CH(CF_3)CH_2CO] unit as a surrogate for the malonyl moiety featured in conventional PM retropeptides (Figure 1).[5]

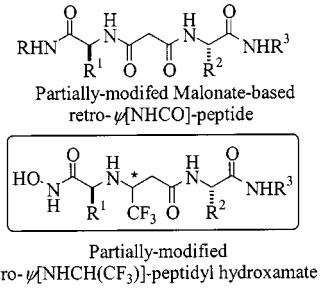

Figure 1. Conventional PM retro-peptides and Tfm-retro-peptidyl hydroxamates

Results and Discussion

Solution-Phase Synthesis

Amino acid derived *O*-Bn hydroxamates **1a−c** were prepared by 1-hydroxybenzotriazole/diisopropylcarbodiimide-

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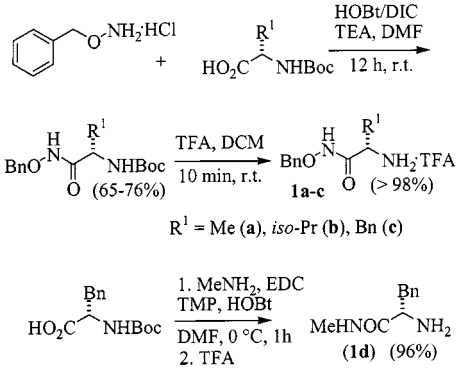
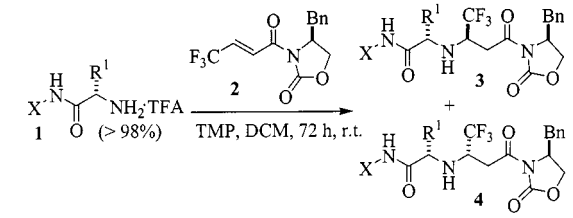
promoted (HOBt/DIC-promoted) condensation of *N*-Boc-α-amino acids with *O*-Bn-hydroxylamine hydrochloride in DMF/triethylamine (TEA), followed by *N*-Boc cleavage with trifluoroacetic acid (TFA) in dichloromethane (DCM) (Scheme 1). The methylamide H-Phe-NHMe (**1d**) was analogously prepared by condensation of Boc-Phe-OH with methylamine hydrochloride, followed by the usual Boc cleavage.



Scheme 1

The PM retro-ψ[NHCH(CF3)]-peptidyl hydroxamate backbone was constructed by means of a Michael-type *N*-addition between **1a−d** and the chiral 3-[(*E*)-enoyl]-1,3-oxazolidin-2-one **2** (Scheme 2 and Table 1).[6] The diastereomeric hydroxamates **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 room temp. The addition of H-Phe-NHMe (**1d**) to **2** was also found to produce the diastereomeric amides **3** and **4d** cleanly. Excess **2** could be recovered quantitatively by flash chromatography (FC). These reactions were very clean and high-yielding, although low diastereocontrol was achieved in all cases. From the perspective of combinatorial application, however, the low stereocontrol is not necessarily a drawback, because both epimers at the trifluoromethyl-substituted (Tfm-substituted) stereocentre can be produced. Diastereomerically pure **3** and **4a−d** were isolated by FC. These Michael-type additions with α-amino hydroxamates and amides **1a−d** are notably less stereoselective than those involving the corresponding α-amino esters.[5a][5b] For example, L-Val-OBn reacted with **2** to provide an 86:14 ratio in favour of the **4b** analogue. The reasons for this rather surprising drop in stereoselectivity are unclear, but we have found that steric hindrance by the CO_2R group has a minor influence on stereocontrol of α-amino ester additions. In the case of L-Phe-OR, in fact, nearly the same degree of stereoselectivity was observed when R = benzyl or *tert*-butyl. This suggests that the low stereocontrol observed in this work may be due to the fact that hydroxamates/amides such as **1** react with **2** through different, less stereoselective conformations than those adopted by α-amino esters.

Exocyclic cleavage of the oxazolidin-2-one ring was performed by treatment of **4a**, **4b**, and **3a** with lithium hydroperoxide generated in situ (Scheme 3),[7] which delivered the expected acids **5a−c** without affecting the hydroxamate moiety. The target PM retro-tripeptidyl hydroxamates **7a**, **7b**, and **7c** were obtained through HATU/HOAt-promoted coupling[8] with L-Val-OBn (**6a**), L-Phe-O-*t*Bu (**6b**), and D-Ala-OMe (**6c**), respectively, followed by catalytic hydrogenolysis of the terminal OBn groups. The methyl ester **7c**, initially isolated by FC in purity > 98%, was found to be unstable even at 4 °C, producing complex mixtures of products within a few days, while the acid **7a** and the *t*Bu ester **7b** were perfectly stable under the same storage conditions.

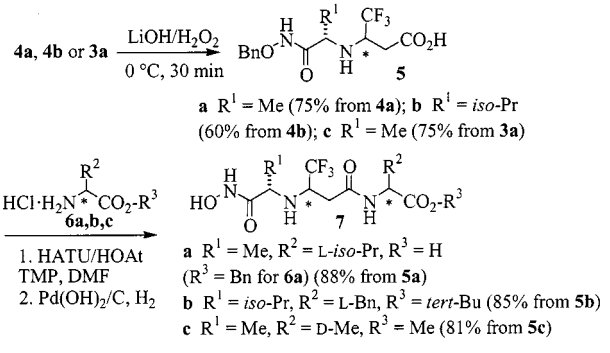


Scheme 2

Table 1. Conjugate additions of **1a−d** to **2**

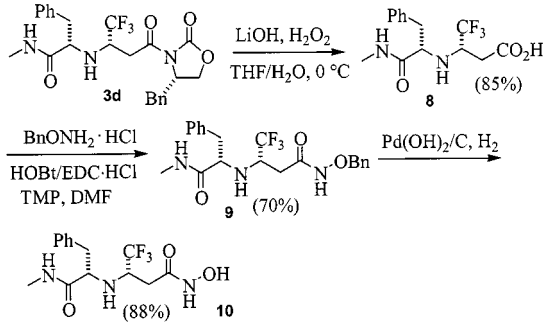
Entry	Products	R^1	X	Ratio **3/4**[a]	Yield (%)[b]
1	**3a** and **4a**	Me	OBn	1.0:1.2	94
2	**3b** and **4b**	*i*Pr	OBn	1.0:2.0	97
3	**3c** and **4c**	Bn	OBn	1.5:1.0	> 98
4	**3d** and **4d**	Bn	Me	1.7:1.0	83

[a] Determined by ^1H and ^{19}F NMR of the crude reaction mixture. [b] Isolated yields.



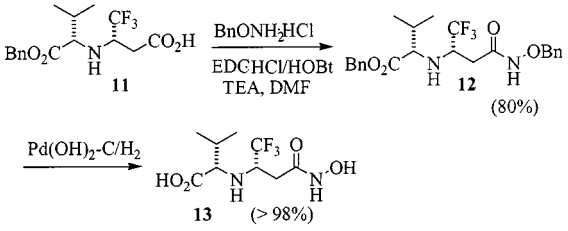
Scheme 3

A similar strategy was applied on the methylamide **3d** (Scheme 4), which was treated with lithium hydroperoxide to provide the acid **8**. Coupling of **8** with *O*-Bn hydroxylamine provided **9**, which was hydrogenolysed to the free hydroxamate **10** in good overall yields.



Scheme 4

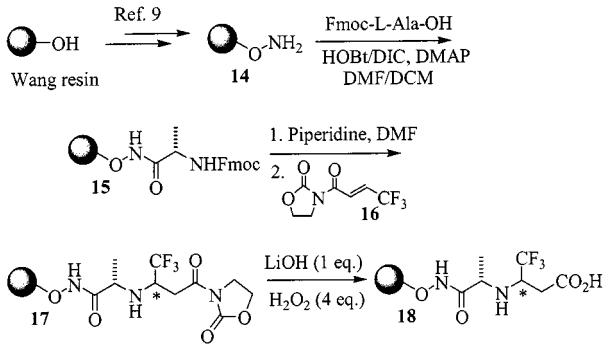
In order to prepare further PM retro-ψ[NHCH(CF_3)]-peptidyl hydroxamates with structures closely resembling those of known bioactive hydroxamates, we conceived of the preparation of another molecule (**13**), with the hydroxamate function directly bound to the CF_3-malonyl mimetic (Scheme 5). We started from the retro-ψ[NHCH(CF_3)]-dipeptide **11**,[5a] easily available by condensation of L-Val-OBn to **2** followed by the usual oxazolidin-2-one cleavage. This was coupled to $BnONH_2$ to give the BnO-hydroxamate **12**, which was directly transformed into **13** by simultaneous hydrogenolysis of the two terminal OBn moieties.



Scheme 5

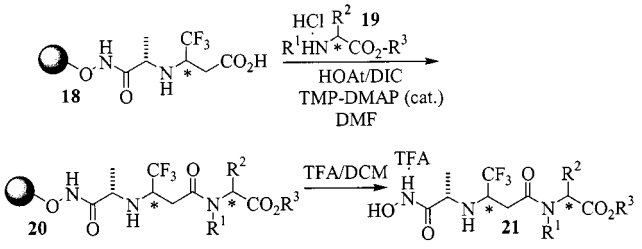
Solid-Phase Synthesis

This method was also adapted to solid-phase conditions, with a view to the preparation of combinatorial libraries of PM retro-ψ[NHCH(CF_3)]-peptidyl hydroxamates for screening as metalloprotease inhibitors. Firstly, we addressed the preparation of retro-tripeptidyl hydroxamates **21a–j** and **23a–d** (see Schemes 7 and 8). The hydroxylamine resin **14** (Scheme 6) was prepared in two steps from commercial Wang resin, according to the method of Floyd,[9] and coupled to an excess of L-Fmoc-Ala to give the protected alanine polymer **15**, 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 **16** (3 equiv. in DCM, 3 d, room temp.). The FT-IR spectrum of the resulting Tfm-resin **17** exhibited a strong O(CO)N band at 1785 cm^{-1}, absent in the precursor **15**. Treatment of **17** with lithium hydroxide (1 equiv. based on the theoretical loading) and hydrogen peroxide (4 equiv.) in THF/H_2O (0 °C, 2 h) cleaved the oxazolidin-2-one with excellent chemoselectivity. As a result, the FT-IR spectrum of the CO_2H resin **18** showed the disappearance both of the O(CO)N band at 1785 cm^{-1} and of the amide band (1700 cm^{-1}) with formation of an intense band at 1620 cm^{-1}, attributable to the carboxyl residue.



Scheme 6

Coupling of **18** to α-amino esters **19** (DIC/HOAt, TMP-DMAP[10]) (Scheme 7 and Table 2) afforded the tripeptidyl resins **20**, from which the retro- and retro-inverso-hydroxamates **21a–j** were released in good yields and purity upon treatment with TFA/DCM (1 h, room temp.).[11] As expected, ^1H and ^{19}F NMR spectroscopy showed that **21a–j** were formed as nearly equimolar mixtures of epimers at the Tfm-substituted centre. For R^3 = *tert*-butyl (Entries 9,10), TFA treatment resulted in concomitant hydrolysis of the terminal ester function, delivering the free carboxyl derivatives **21i** and **21j**, which were found to be indefinitely stable upon storage at 4 °C.



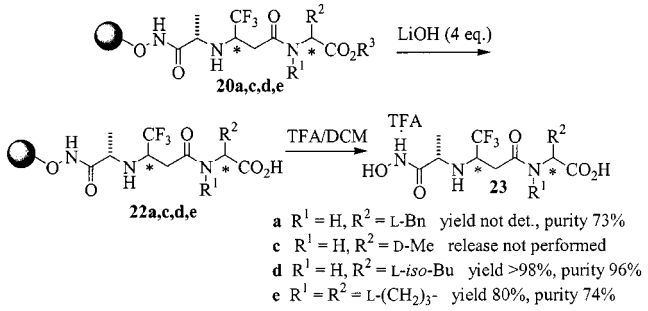
Scheme 7

Table 2. Solid-phase synthesis of Tfm-retro-peptidyl hydroxamates **21**

Entry	Product	R^1	R^2	R^3	Yield (%)	Purity (%)[a]
1	**21a**	H	L-Bn	Bn	n.d.	73
2	**21b**	H	L-Me	Me	76	100
3	**21c**	H	D-Me	Me	54	73
4	**21d**	H	L-*i*Bu	Bn	75	55
5	**21e**	L-$(CH_2)_3$		Bn	72	55
6	**21f**	H	L-*i*Pr	Bn	58	57
7	**21g**	H	H	Me	n.d.	89
8	**21h**	H	L-*s*Bu	Me	62	65
9	**21i**	H	H	H[b]	60	87
10	**21j**	H	L-Me	H[b]	60	79

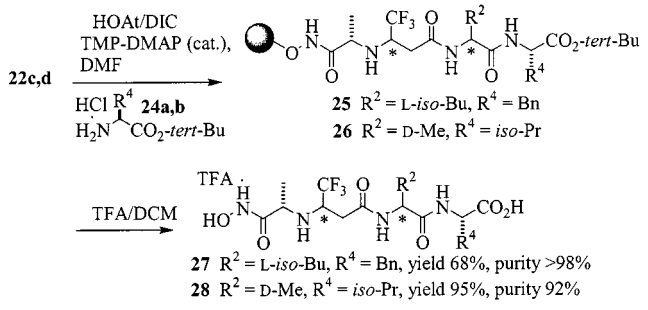
[a] Determined by ^1H and ^{19}F NMR after 24–48 h at 4 °C. [b] R^3 = *t*Bu for **19**, **20i**, **20j**.

In contrast, the hydroxamates **21a−h**, with terminal benzyl or methyl ester functions, were found to be rather unstable under the same storage conditions, similarly to compound **7c** prepared in solution. As a consequence, moderate to low purity of the samples was measured by ^{1}H and ^{19}F NMR after 24−48 h at 4 °C (Table 2), while nearly complete conversion of **21a−h** into unidentified by-products took place after an additional 2−3 d at the same temperature. In order to prepare further stable hydroxamates by solid-phase synthesis, the ester functions of polymers **20a**, **20d**, and **20e** (Scheme 8) were first hydrolysed with lithium hydroxide to give **22a**, **22d**, and **22e**, after which TFA treatment of the resins released the stable, free carboxyl hydroxamates **23a**, **23d**, and **23e**, with very good overall yields and purities. Hydrolysis of the di-alanyl methyl ester resin **20c** to the acid **22c** was also performed satisfactorily, according to IR analysis.

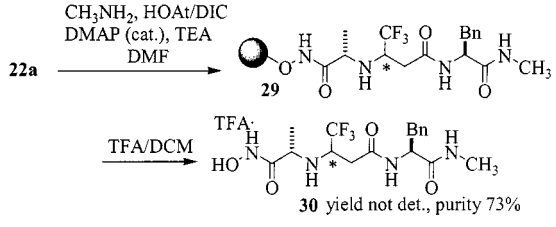


Scheme 8

Next, in order to demonstrate the versatility of the method, we addressed the solid-phase preparation of tetrapeptidyl hydroxamates possessing CO_2H termini (**27** and **28**) (Scheme 9), as well as a tripeptidyl hydroxamate with a methylamide terminus (**30**), often encountered in metalloprotease inhibitors (Scheme 10).[1] Tripeptidyl hydroxamate



Scheme 9



Scheme 10

polymers **22c** and **22d**, each possessing a CO_2H endgroup (Scheme 9), were coupled to L-Phe-O*t*Bu (**24a**) and L-Val-O*t*Bu (**24b**) (HOAt/DIC, TMP-DMAP), to afford the resin-bound tetrapeptidyl retro and retro-inverso derivatives **25** and **26**, respectively. The free hydroxamates **27** and **28** were released with TFA/DCM in excellent purity as mixtures of two epimers. Finally, the retro-tripeptidyl hydroxamic amide **30** (Scheme 10) was prepared in good purity upon coupling of the polymer **22a** to methylamine (HOAt/DIC, TEA-DMAP), followed by the usual release from **29** with TFA/DCM.

Stereochemical Assignments

The absolute stereochemistry of hydroxamates **3** and **4** and their derivatives was unambiguously assigned by a combination of X-ray diffraction and chemical correlation with known ψ[NHCH(CF_3)]-retropeptides.[5a] X-ray diffraction was performed on a suitable single crystal of **4b**, which showed very interesting conformational properties. A view of **4b** is shown in Figure 2 and selected molecular dimensions are reported in Table 3. Bond lengths and angles fall in the expected ranges.[12] All the values of the bond angles on N(3), including the hydrogen atom located by a Fourier difference map, indicate a nearly planar geometry at N(3), as commonly found in amides. Closely similar, although more surprising conclusions, can be reached with respect to the flattened geometry of the amine nitrogen atom N(2). This effect may result from hyperconjugation involving the fluorine atoms of the adjacent Tfm group or from steric interactions between the bulky substituents on N(2).

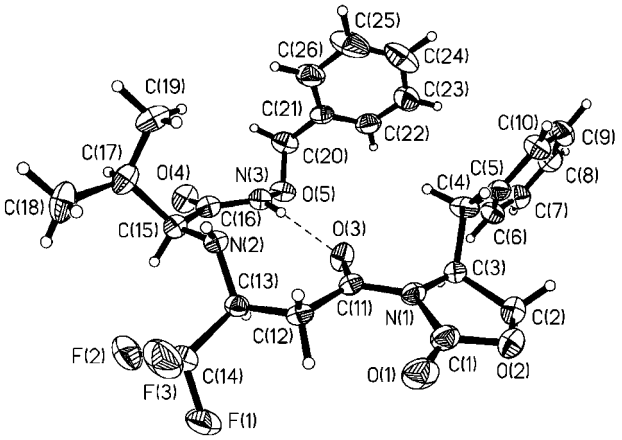


Figure 2. ORTEP view of **4b**, showing the absolute configuration and the atomic labelling scheme; 20% thermal ellipsoids are shown for non-hydrogen atoms

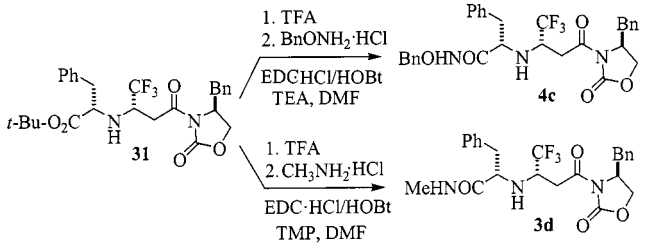
An intramolecular hydrogen bond, giving rise to a nine-membered ring, is found between the amide hydrogen atom on N(3) and oxygen atom O(3) [O···H 1.95(3) Å; O···H−N 157.4(2)°]. The eclipsed conformations on the C(15)−C(16) and the C(12)−C(11) bonds, and the gauche conformation on the C(12)−C(13) bond, suggest that the molecule deforms to favour the intramolecular interaction with the amide (and hence more acidic) hydrogen atom. It is worth

Table 3. Selected molecular dimensions for **4b**

Bond lengths	[Å]	Bond angles	[°]
N(1)−C(1)	1.384(4)	C(15)−N(2)−C(13)	119.2(2)
N(1)−C(11)	1.390(3)	C(16)−N(3)−O(5)	117.7(3)
N(1)−C(3)	1.470(3)	C(1)−N(1)−C(11)	127.3(3)
N(2)−C(13)	1.449(3)	C(1)−N(1)−C(3)	111.3(2)
N(2)−C(15)	1.462(3)	C(11)−N(1)−C(3)	120.3(2)
N(3)−C(16)	1.328(4)	N(1)−C(11)−C(12)	118.5(2)
N(3)−O(5)	1.401(3)	C(11)−C(12)−C(13)	113.5(2)
O(2)−C(1)	1.340(4)	N(2)−C(13)−C(12)	112.03(2)
O(2)−C(2)	1.429(5)	N(2)−C(13)−C(14)	113.5(2)
O(3)−C(11)	1.212(3)	C(12)−C(13)−C(14)	107.4(3)
O(4)−C(16)	1.233(4)	N(2)−C(15)−C(16)	112.6(2)
O(5)−C(20)	1.437(4)		
F(1)−C(14)	1.332(4)	Torsion angles	[°]
F(2)−C(14)	1.307(4)	C(13)−N(2)−C(15)−C(16)	−94.3(3)
F(3)−C(14)	1.338(5)	C(15)−N(2)−C(13)−C(12)	163.0(2)
C(2)−C(3)	1.518(4)	C(11)−C(12)−C(13)−N(2)	−71.6(3)
C(3)−C(4)	1.515(3)	N(2)−C(15)−C(16)−N(3)	−6.6(4)
C(11)−C(12)	1.482(4)	O(3)−C(11)−C(12)−C(13)	5.1(4)
C(12)−C(13)	1.522(4)	C(16)−N(3)−O(5)−C(20)	92.0(3)
C(13)−C(14)	1.531(4)	N(3)−O(5)−C(20)−C(21)	71.4(4)
C(15)−C(16)	1.522(4)	O(5)−N(3)−C(16)−C(15)	171.5(2)
C(15)−C(17)	1.539(4)	C(11)−N(1)−C(3)−C(4)	78.7(3)

noting that Gellman et al. reported evidence of similar turn-like secondary structures in several nonfluorinated small retro-peptides.[13] An additional, weaker intermolecular hydrogen bond involving the amine hydrogen atom H(N2) and O(4) as acceptor [O(4)···H(N2) 2.29(3) Å; O(4)···H(N2)−N(2) 175(3)°] probably plays a significant role in the crystal packing. The propensity of H(N2) to form hydrogen bonds − i.e., its acidity − may well be increased by the nearby trifluoromethyl group. Another significant intramolecular interaction occurs between phenyl groups, as indicated by the relative orientation of the two rings (ca. 40° dihedral angle between the ring planes) and by the relatively short distance (around 3.5 Å) between atoms of the two systems.

In order to complete the stereochemical assignments, compound **31** (Scheme 11), of known configuration,[5a] was transformed both into the benzyl hydroxamate **4c** and into the amide **3d**, providing a safe assignment for molecules **3**, **4c**, and **4d**. In the remaining case (**a**), the absolute configuration was tentatively assigned by comparison of the R_f values of **3** and **4a** with those of **3b**, **3c**, and **3d** (with lower R_f values) and **4b**, **4c**, and **4d** (with higher R_f values).



Scheme 11

Biological Assays on MMP-9

To study the effect of some Tfm-hydroxamates on MMP-9 (gelatinase B) expression, we incubated human monocyte derived macrophages with compounds **10**, **13**, and **30** for 24 h. The conditioned media were then collected and analysed by gelatin-zymography to evaluate the potential gelatinolytic capacity of MMP-9. As shown in Figure 3, the compound **13** significantly inhibited MMP-9 gelatinolytic capacity in a concentration-dependent manner at 10 and 100 μM (−27% $P < 0.05$; and −43% $P < 0.01$, respectively). Compound **10** displayed an inhibitory effect only at the concentration of 100 μM (−30%, $P < 0.05$), while compound **30** did not have any effect on MMP-9 activity in human macrophages.

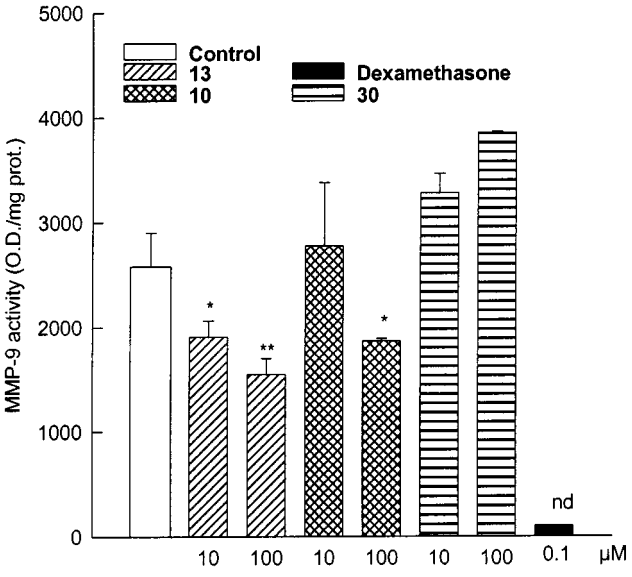


Figure 3. Effect of compounds on MMP-9 activity in human macrophages; student's test: * $P < 0.05$, ** $P < 0.01$ vsersus control

To check whether the inhibitory effect of the compounds was also due to direct interference with the activation process of MMP-9, aliquots of conditioned media obtained after incubation of human macrophages with DMEM alone were examined by electrophoresis on gelatin-containing gels. The gels were then cut into strips and the compounds were added during the overnight activation step. The data reported in Figure 4 show that only compound **30**, at the highest concentration tested, displayed a statistically significant inhibitory effect under these experimental conditions (−44%, $P < 0.01$), suggesting a direct interaction with the proteinase, affecting its activation. Compound **10** showed an inhibitory trend, but it did not reach a significant threshold. Compound **13** was completely inactive by this parameter.

This demonstrates that the in vitro incubation of human macrophages with **13** and **10** reduced MMP-9 (gelatinase B) total potential gelatinolytic capacity in gelatin-zymography. This effect is probably due to an inhibitory effect on pro-

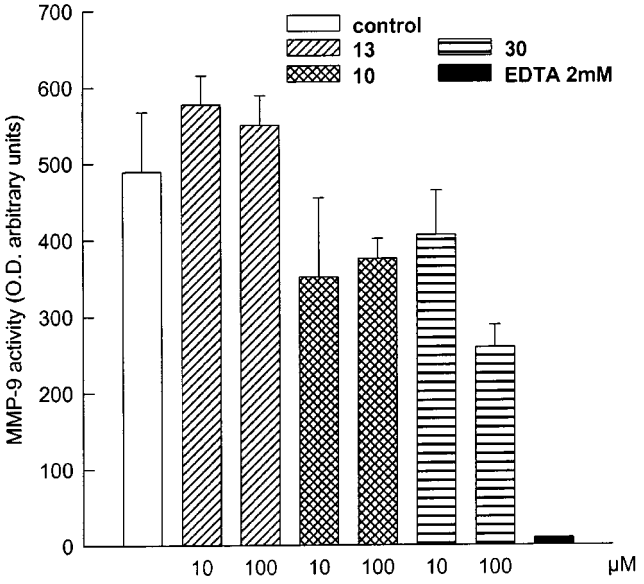


Figure 4. Effect of compounds on the activity of MMP-9 already secreted by cells; student's test: * $P < 0.01$ versus control

teinase release from cells by the compounds. In contrast, our data indicate that compound **30** has an effect that is consistent with direct inhibition of proteinase activity.

Conclusions

In summary, we have developed both solution and solid-phase procedures for preparation of a novel structural family of fluorinated retro-peptidyl hydroxamates. Although we did not in this work achieve a true combinatorial approach to the title compounds, the examples shown provide good evidence that the method is general and could be applied for that purpose. X-ray analysis of the Tfm-hydroxamate **4b** showed an interesting turn-like conformation and a surprising quasi-planar geometry of the NH group bound to the Tfm-substituted carbon atom, which mimics a retro-peptidic NH. Bioassays on randomly chosen Tfm-retropeptidyl hydroxamates **10**, **13**, and **30** showed reduction of the gelatinolytic capacity of MMP-9, suggesting that a high-throughput screening of libraries of these novel hydroxamates might evidence some remarkably active hit. These issues are at present being addressed in our laboratories.

Experimental Section

General: Chemical shifts (δ) are reported in ppm of the applied field. Coupling constants (*J*) are reported in Hz. Me_4Si was used as internal standard ($\delta_H = \delta_C = 0.00$) for ^1H and ^{13}C nuclei, while C_6F_6 was used as external standard ($\delta_F = -162.90$) for ^{19}F nuclei. Peak multiplicities are abbreviated: singlet, s; doublet, d; triplet, t; quadruplet, q; multiplet, m, etc. A three-stage DIS (Direct Inlet System) quadrupole instrument was used for mass spectrometry of pure compounds. Anhydrous solvents were obtained by distillation from sodium (THF, benzene) or from calcium hydride (dichloromethane, diisopropylamine). In all other cases, commercially available reagent-grade solvents were employed without purification. Reactions performed in dry solvents were carried out under nitrogen. Melting points are uncorrected and were obtained with a capillary apparatus. Analytical thin layer chromatography (TLC) was routinely used to monitor reactions in solution. Plates precoated with E. Merck 60 F_{254} silica gel of 0.25 mm thickness were used. Merck 60 silica gel (230−400 ASTM mesh) was employed for flash chromatography (FC). Wang resin (100−200 mesh, loading 1.3 mmol/g) was purchased from Novabiochem. Amino acid derived BnO-hydroxamates **1a**,[14] **1b**,[14] **1c**,[15] methylamide **1d**,[16] hydroxylamine resin **14**,[9] and Tfm-oxazolidin-2-ones **2** and **16**[6] were prepared according to literature methods.

Solution-Phase Michael Addition. − Synthesis of Compounds 3a−d and 4a−d. − Typical Procedure: Neat *sym*-collidine (0.48 mL, 3.6 mmol) was added to a solution of **1a** (369.9 mg, 1.2 mmol) and **2** (1.05 g, 3.6 mmol) in CH_2Cl_2 (15 mL). After 72 h at room temp. the solvent was removed in vacuo and the crude material was dissolved in EtOAc and washed once with 1 N HCl. The organic layer was dried with anhydrous Na_2SO_4 and filtered, the solvent was removed in vacuo, and the crude material was purified by FC (hexane/EtOAc, 70:30) to afford 306 mg of the major diastereoisomer **4a**, 249 mg of the minor diastereoisomer **3a** (94% overall) and 704 mg of unchanged Michael acceptor **2**.

Compound 3a: Oil; $R_f = 0.14$ (hexane/EtOAc, 60:40). $[\alpha]_D^{23} = +20.7$ ($c = 0.8$, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$): δ = 9.40 (br. s, 1 H), 7.42−7.15 (m, 10 H), 4.91 (d, $J = 11.5$, 1 H), 4.88 (d, $J = 11.5$, 1 H), 4.65 (m, 1 H), 4.16 (m, 2 H), 3.74 (m, 1 H), 3.41 (q, $J = 6.9$, 1 H), 3.29 (m, 2 H), 3.16 (dd, $J = 17.9$, 7.8, 1 H), 2.75 (dd, $J = 13.7$, 9.6, 1 H), 2.28 (br. s, 1 H), 1.35 (d, $J = 6.9$, 3 H). ^{19}F NMR (500 MHz, $CDCl_3$): δ = −76.0 (d, $J = 7.4$).

Compound 4a: Solid; $R_f = 0.40$ (hexane/EtOAc, 60:40). $[\alpha]_D^{23} = +39.2$ ($c = 0.8$, $CHCl_3$); m.p. 155−156 °C. FTIR (KBr): ν_{max} = 3311, 3251, 1791, 1782, 1702, 1228, 1146 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ = 10.00 (br. s, 1 H), 7.40−7.15 (m, 10 H), 4.98 (d, $J = 11.0$, 1 H), 4.92 (d, $J = 11.0$, 1 H), 4.70 (m, 1 H), 4.27 (m, 1 H), 4.18 (dd, $J = 8.7$, 2.3, 1 H), 3.64 (q, $J = 6.9$, 1 H), 3.59 (m, 1 H), 3.22 (m, 2 H), 3.12 (dd, $J = 17.4$, 10.5, 1 H), 2.74 (dd, $J = 13.3$, 9.6, 1 H), 1.77 (br. s, 1 H), 1.34 (d, $J = 6.9$, 3 H). ^{19}F NMR (500 MHz, $CDCl_3$): δ = −74.6 (d, $J = 6.0$). ^{13}C NMR (125.6 MHz, $CDCl_3$): δ = 171.1, 170.1, 153.2, 135.4, 134.8, 129.4, 129.3, 129.0, 128.6, 128.4, 127.5, 126.1 (q, $J = 283.5$), 77.8, 66.6, 55.3, 55.2, 54.3 (q, $J = 28.2$), 37.8, 36.0, 20.3. MS (70 eV); *m/z* (%): 494 (13) [$M^+ + 1$], 343 (100), 91 (63).

Compound 3b: $R_f = 0.50$ (hexane/EtOAc, 60:40). ^1H NMR (500 MHz, $CDCl_3$): δ = 9.02 (br. s, 1 H), 7.43−7.18 (m, 10 H), 4.95 (d, $J = 11.5$, 1 H), 4.91 (d, $J = 11.5$, 1 H), 4.68 (m, 1 H), 4.18 (m, 2 H), 3.72 (m, 1 H), 3.28 (m, 3 H), 2.76 (dd, $J = 13.8$ and 9.9, 1 H), 1.27 (m, 1 H), 0.96 (d, $J = 6.9$, 3 H), 0.91 (d, $J = 6.9$, 3 H). ^{19}F NMR (500 MHz, $CDCl_3$): δ = −76.0 (d, $J = 7.6$).

Compound 4b: Solid; $R_f = 0.63$ (hexane/EtOAc, 60:40). $[\alpha]_D^{23} = +30.1$ ($c = 0.6$, $CHCl_3$); m.p. 111−112 °C. FTIR (KBr): ν_{max} = 3315, 3240, 1795, 1781, 1702, 1255, 1126 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ = 9.86 (br. s, 1 H), 7.40−7.15 (m, 10 H), 4.97 (d, $J = 11.2$, 1 H), 4.91 (d, $J = 11.2$, 1 H), 4.72 (m, 1 H), 4.26 (m, 1 H), 4.14 (dd, $J = 8.6$, 2.5, 1 H), 3.57 (m, 1 H), 3.27 (m, 3 H), 3.14 (dd, $J = 16.6$, 10.4, 1 H), 2.77 (dd, $J = 13.3$, 9.7, 1 H), 2.03 (m, 1 H), 1.70 (br. s, 1 H), 0.96 (d, $J = 6.8$, 3 H), 0.90 (d, $J = 6.8$, 3 H). ^{19}F NMR (500 MHz, $CDCl_3$): δ = −75.5 (d, $J = 6.2$). ^{13}C NMR (125.6 MHz, $CDCl_3$): δ = 170.3, 169.8, 153.3, 135.0, 129.3, 129.2, 129.0, 128.9, 128.5, 128.3, 127.3, 126.1 (q, $J = 285.1$),

78.0, 66.6, 64.8, 55.3, 54.3 (q, J = 27.9), 37.6, 36.1, 31.6, 19.0, 17.5. MS (70 eV); m/z (%): 522 (14) [M^+], 371 (100), 91 (55).

Crystal Data for 4b: $C_{26}H_{30}F_3N_3O_5$, formula mass 521.53, orthorhombic, space group $P2_12_12_1$, a = 6.184(0) Å, b = 18.476(1) Å, c = 23.256(1) Å, V = 2657.1(5) Å^3, Z = 4, D_c = 1.304 g/cm^3, μ = 0.887 mm^{-1}, $F(000)$ = 1096. Data collection: X-ray diffraction data were collected from a colourless prismatic crystal of **4b** (size 0.55 × 0.35 × 0.25 mm) with a Siemens P4 diffractometer (θ-2θ scan technique), with graphite-monochromated Cu-K_α radiation (λ = 1.5418 Å). 3353 reflections were collected ($3.05° < \theta < 67.76°$; $+h,+k,+l$ and $-h,-k,-l$), 3145 unique reflections, 3 standard reflections, measured every 100 reflections, showed no decay. Data were corrected for Lorentz and polarization effects and an empirical absorption correction was applied. Structure analysis and refinement: The structure was solved by direct methods with SIR92[17] and refined by full-matrix, least-squares on F^2 with SHELXL97.[18] Non-hydrogen atoms were refined anisotropically. The *N*-bonded hydrogen atoms were located by difference Fourier techniques and refined, while all the others were included at calculated positions and refined with group temperature factors. Final values of the residual $R1$ for reflections with $I > 2\sigma$ and for all reflections were 0.037 and 0.046, respectively. The highest peak and hole in the final difference-Fourier map were 0.128 and −0.106 e·Å^3. The refined structure unequivocally indicates that all the three asymmetric carbon atoms share the same configuration. The correct absolute configuration, as implied by chemical correlation, is also suggested by the refinement, albeit with very low reliability. The value of the Flack parameter[19] is 0.3(2). Crystallographic data (excluding structure factors) for structure **4b** reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-167166. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [Fax: (internat.) + 44-1223/336-033; E-mail: deposit@ccdc.cam.ac.uk].

Compound 3c: Overall yield > 98%, oil; R_f = 0.59 (hexane/EtOAc, 60:40). $[\alpha]_D^{23}$ = + 8.1 (c = 0.7, $CHCl_3$). FTIR (KBr): ν_{max} = 3377, 3216, 1777, 1687, 1202, 1111cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 9.29 (br. s, 1 H), 7.37−7.13 (m, 15 H), 4.86 (s, 2 H), 4.53 (m, 1 H), 4.14 (m, 2 H), 3.69 (m, 1 H), 3.59 (m, 1 H), 3.19 (m, 2 H), 3.05 (dd, J = 17.9, 4.5, 1 H), 2.87 (dd, J = 17.9, 7.4, 1 H), 2.81 (dd, J = 13.4, 8.2, 1 H), 2.67 (dd, J = 13.4, 9.7, 1 H), 2.05 (br. s, 1 H). ^{19}F NMR (500 MHz, $CDCl_3$): δ = −76.4 (d, J = 8.7). ^{13}C NMR (125.6 MHz, $CDCl_3$): δ = 169.7, 168.9, 153.1, 136.6, 135.0, 134.9, 129.4, 129.2, 129.0, 128.8, 128.7, 128.6, 127.5, 125.6 (q, J = 281.1), 78.2, 66.4, 61.3, 55.23 (q, J = 29.6), 55.20, 39.5, 37.6, 35.3. MS (70 eV); m/z (%): 570 (39) [M^+ + 1], 419 (38), 91 (100).

Compound 4c: Oil; R_f = 0.76 (hexane/EtOAc, 50:50). $[\alpha]_D^{23}$ = −8.8 (c = 0.7, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 9.86 (br. s, 1 H), 7.35−7.13 (m, 15 H), 4.93 (d, J = 11.5, 1 H), 4.87 (d, J = 11.5, 1 H), 4.66 (m, 1 H), 4.24 (m, 1 H), 4.15 (dd, J = 9.2, 2.7, 1 H), 3.82 (m, 1 H), 3.56 (m, 1 H), 3.16 (m, 3 H), 3.00 (dd, J = 17.4, 10.5, 1 H), 2.89 (dd, J = 13.7, 7.3, 1 H), 2.70 (dd, J = 13.7, 9.6, 1 H), 1.89 (br. s, 1 H). ^{19}F NMR (500 MHz, $CDCl_3$): δ = −74.3 (d, J = 6.1). ^{13}C NMR (125.6 MHz, $CDCl_3$): δ = 170.1, 169.8, 153.2, 136.1, 135.4, 134.8, 129.3, 129.2, 129.0, 128.9, 128.6, 128.4, 127.5, 127.3, 125.9 (q, J = 285.8), 78.0, 66.6, 60.3, 55.2, 54.2 (q, J = 28.2), 39.6, 37.7, 36.1.

Compound 3d: Overall yield 83%, R_f = 0.22 (Et_2O/hexane, 80:20). $[\alpha]_D^{23}$ = −44.3 (c = 0.5, $CHCl_3$). FTIR (KBr): ν_{max} = 3369, 3223, 1772, 1679 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.36−7.16 (m, 10 H), 4.70 (m, 1 H), 4.25 (m, 1 H), 4.18 (dd, J = 8.7, 2.7, 1 H), 3.83 (dd, J = 7.8, 5.0, 1 H), 3.70 (m, 1 H), 3.26 (dd, J = 13.3, 3.2, 1 H), 3.20−3.09 (m, 3 H), 2.87 (dd, J = 13.7, 8.2, 1 H), 2.81 (d, J = 5.0, 3 H), 2.76 (dd, J = 13.3, 9.2, 1 H), 1.88 (br. s, 1 H). ^{19}F NMR (500 MHz, $CDCl_3$): δ = −73.9 (d, J = 6.9). ^{13}C NMR (125.6 MHz, $CDCl_3$): δ = 173.0, 169.9, 153.2, 136.5, 134.8, 129.3, 129.2, 129.1, 128.8, 127.6, 127.1, 126.0 (q, J = 285.5), 66.6, 61.0, 55.3, 54.2 (q, J = 27.3), 39.6, 37.9, 36.1, 25.8. MS (70 eV); m/z (%): 478 (40) [M^+ + 1], 419 (100), 386 (24).

Compound 4d: R_f = 0.11 (Et_2O/hexane, 80:20). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.40−7.10 (m, 10 H), 4.55 (m, 1 H), 4.18 (m, 2 H), 3.66 (m, 1 H), 3.56 (dd, J = 9.3 and 3.9, 1 H), 3.20 (m, 3 H), 2.84 (d, J = 5.0, 3 H), 2.71 (m, 3 H), 2.15 (br. s, 1 H). ^{19}F NMR (500 MHz, $CDCl_3$): δ = −76.0 (d, J = 6.5).

Solution-Phase Cleavage of the Oxazolidinone Auxiliary. − Synthesis of Compounds 5a−b and 8. − Typical Procedure: A 30% aqueous H_2O_2 solution (0.082 mL, 0.8 mmol) was added at 0 °C under nitrogen to a cooled solution of **3a** (100 mg, 0.20 mmol) in THF/H_2O (4:1) (2 mL), followed by solid LiOH·H_2O (8.5 mg, 0.2 mmol). After 30 min, the reaction mixture was quenched with saturated aqueous Na_2SO_3, allowed to warm to room temperature, diluted with 5% aqueous $NaHCO_3$ and extracted with EtOAc. The aqueous layer was acidified with 1 N HCl and extracted with EtOAc. The organic layers were dried with anhydrous Na_2SO_4 and filtered, and the solvent was removed in vacuo to afford 50 mg of pure acid **5c**.

Compound 5a: Yield 75%, oil; R_f = 0.23 ($CHCl_3$/MeOH, 90:10). $[\alpha]_D^{23}$ = −16.1 (c = 0.8, MeOH). FTIR (film): ν_{max} = 3343, 1738, 1624, 1236 cm^{-1}. ^{1}H NMR (500 MHz, CD_3OD): δ = 7.45 (m, 2 H), 7.34 (m, 3 H), 4.93 (d, J =10.1, 1 H), 4.90 (d, J = 10.1, 1 H), 3.47 (q, J = 6.9, 1 H), 3.41 (m, 1 H), 2.57 (m, 1 H), 1.23 (d, J = 6.9, 3 H). ^{19}F NMR (500 MHz, CD_3OD): δ = −76.5 (d, J = 6.8). ^{13}C NMR (125.6 MHz, CD_3OD): δ = 176.8, 173.7, 137.9, 130.5, 129.6, 129.4, 127.8 (q, J = 283.7), 78.8, 67.8, 56.4 (q, J = 27.8), 55.7, 20.1. MS (70 eV); m/z (%): 335 (43) [M^+ + 1], 184 (83), 91 (100).

Compound 5b: Yield 60%, oil; R_f = 0.33 ($CHCl_3$/MeOH 90:10). $[\alpha]_D^{23}$ = −2.2 (c = 0.8, MeOH). FTIR (film): ν_{max} = 3335, 1730, 1628, 1154 cm^{-1}. ^{1}H NMR (500 MHz, CD_3OD): δ = 7.45 (m, 2 H), 7.35 (m, 3 H), 4.94 (d, J = 10.5, 1 H), 4.89 (d, J = 10.5, 1 H), 3.43 (m, 1 H), 2.96 (d, J = 6.4, 1 H), 2.63 (dd, J = 16.5, 8.2, 1 H), 2.51 (dd, J = 16.5, 8.2, 1 H), 1.82 (m, 1 H), 0.92 (d, J = 6.9, 3 H), 0.89 (d, J = 6.9, 3 H). ^{19}F NMR (500 MHz, CD_3OD): δ = −77.0 (d, J = 7.0). ^{13}C NMR (125.6 MHz, CD_3OD): δ = 175.3, 172.7, 137.0, 130.4, 129.5, 127.9 (q, J = 283.2), 79.0, 66.3, 56.7 (q, J = 28.2), 33.0, 19.6, 18.7. MS (70 eV); m/z (%): 363 (100) [M^+ + 1].

Compound 5c: Yield 75%, oil; R_f = 0.22 ($CHCl_3$/MeOH, 90:10). $[\alpha]_D^{23}$ = +11.5 (c = 0.6, MeOH). FTIR (film): ν_{max} = 3356, 1740, 1619, 1270, 1147 cm^{-1}. ^{1}H NMR (500 MHz, CD_3OD): δ = 7.42 (m, 2 H), 7.35 (m, 3 H), 4.87 (d, J =11.0, 1 H), 4.83 (d, J = 11.0, 1 H), 3.58 (m, 1 H), 3.34 (q, J = 6.9, 1 H), 2.67 (dd, J = 16.0, 4.6, 1 H), 2.50 (dd, J = 16.0, 8.2, 1 H), 1.25 (d, J = 6.9, 3 H). ^{19}F NMR (500 MHz, CD_3OD): δ = −77.6 (d, J = 7.4). ^{13}C NMR (125.6 MHz, CD_3OD): δ = 173.6, 173.3, 136.8, 129.7, 129.4, 127.4 (q, J = 281.6), 79.0, 57.2 (q, J = 29.1), 56.4, 35.1, 20.0. MS (70 eV); m/z (%): 335 (43) [M^+ + 1], 184 (83), 91 (100).

Compound 8: Yield 85%, solid; R_f = 0.24 ($CHCl_3$/MeOH, 90:10). $[\alpha]_D^{23}$ = −57.2 (c = 0.7, MeOH); m.p. 132−133 °C. FTIR (KBr): ν_{max} = 3354, 1735, 1625, 1265, 1143 cm^{-1}. ^{1}H NMR (500 MHz, CD_3OD): δ = 7.30−7.18 (m, 5 H), 3.70 (m, 1 H), 3.55 (m, 1 H),

3.00 (dd, J = 13.7, 5.0, 1 H), 2.79 (dd, J = 13.7, 8.2, 1 H), 2.70 (s, 3 H), 2.65 (dd, J = 16.5, 3.7, 1 H), 2.45 (dd, J = 16.5, 9.6, 1 H). ^{19}F NMR (500 MHz, CD_3OD): δ = −76.1 (d, J = 7.0). ^{13}C NMR (125.6 MHz, CD_3OD): δ = 176.2, 173.7, 138.4, 130.3, 129.5, 127.9, 127.8 (q, J = 284.4), 63.2, 56.1 (q, J = 27.9), 48.9, 41.0, 26.0. MS (70 eV); *m*/*z* (%): 319 (7) [M^+ + 1], 260 (100), 200 (21).

Solution-Phase Coupling with α-Amino Esters 6a,b,c. − Typical Procedure: Neat *sym*-collidine (0.032 mL, 0.24 mmol),was added at 0 °C under nitrogen to a stirred solution of **5a** (29 mg, 0.08 mmol) and **6a** (20 mg, 0.08 mmol) in dry DMF (1 mL), followed by solid HOAt (11 mg, 0.08 mmol) and solid HATU (31 mg, 0.08 mmol). After 40 min, the solution was quenched with 1 N HCl, allowed to warm to room temperature, and extracted with EtOAc. The collected organic layers were dried with anhydrous Na_2SO_4 and filtered, the solvent was removed in vacuo, and the crude material was purified by FC (hexane/EtOAc, 50:50), to afford 42 mg of the desired product.

Solution-Phase Coupling with BnO-Hydroxylamine. − Synthesis of Compounds 9 and 12. − Typical Procedure: Neat TEA (0.041 mL, 0.29 mmol) was added at 0 °C under nitrogen to a stirred solution of **11** (23 mg, 0.066 mmol) and BnO-hydroxylamine·HCl (12 mg, 0.069 mmol) in dry DMF (1 mL), followed by solid EDC·HCl (14 mg, 0.073 mmol) and solid HOBt (10 mg, 0.073 mmol). After 12 h, the solution was quenched with 1 N HCl, allowed to warm to room temperature and extracted with EtOAc. The collected organic layers were dried with anhydrous Na_2SO_4 and filtered, the solvent was removed in vacuo, and the crude material was purified by FC (hexane/EtOAc, 80:20) to afford 23 mg of the product **12**.

Compound 9: Yield 70%, R_f = 0.65 ($CHCl_3$/MeOH, 90:10). $[\alpha]_D^{23}$ = −42.5 (c = 0.6, acetone). FTIR (KBr): ν_{max} = 3322, 1649, 1177 cm^{-1}. ^{1}H NMR (500 MHz, [D_6]acetone): δ = 10.59 (br. s, 1 H), 7.48−7.17 (m, 10 H), 4.91 (s, 2 H), 3.72 (m, 1 H), 3.66 (m, 1 H), 3.02 (dd, J = 13.7, 8.7, 1 H), 2.80 (dd, J = 13.7, 7.8, 1 H), 2.71 (d, J = 4.1, 3 H), 2.49 (dd, J = 15.1, 3.2, 1 H), 2.30 (dd, J = 15.1, 10.1, 1 H). ^{19}F NMR (500 MHz, [D_6]acetone): δ = −75.4 (d, J = 6.3, 3 F). ^{13}C NMR (125.6 MHz, [D_6]acetone): δ = 173.8, 167.4, 138.6, 137.1, 130.1, 129.9, 129.2, 129.1, 127.6 (q, J = 284.8), 127.4, 78.5, 62.7, 55.7 (q, J = 28.7), 40.8, 33.9, 26.0. MS (70 eV); *m*/*z* (%): 365 (100) [M^+ − Ph], 332 (23), 91 (64).

Compound 12: Yield 80%, solid; R_f = 0.79 (hexane/EtOAc, 50:50). $[\alpha]_D^{23}$ = +6.3 (c = 0.6, acetone); mp: 104−105 °C. FTIR (KBr): ν_{max} = 3315, 1713, 1668, 1265, 1114 cm^{-1}. ^{1}H NMR (500 MHz, [D_6]acetone): δ = 10.30 (br. s, 1 H), 7.48−7.28 (m, 10 H), 5.22 (d, J = 12.2, 1 H), 5.17 (d, J = 12.2, 1 H), 3.67 (m, 1 H), 3.28 (m, 1 H), 2.52 (br. d, J = 15.0, 1 H), 2.37 (dd, J = 15.0, 7.9, 1 H), 2.30 (br. s, 1 H), 1.93 (m, 1 H), 0.92 (d, J = 6.5, 3 H), 0.83 (d, J = 6.5, 3 H). ^{19}F NMR (500 MHz, acetone): δ = −77.2 (d, J = 6.2). ^{13}C NMR (62.8 MHz, $CDCl_3$): δ = 175.4, 167.4, 137.8, 130.4, 129.9, 129.8, 129.7, 129.5, 128.0 (q, J =181.1), 79.0, 68.3, 67.6, 57.7 (q, J = 28.7), 34.4, 33.2, 20.0, 18.7. MS (70 eV); *m*/*z* (%): 453 (29) [M^+ + 1], 317 (100), 91 (25).

Solution-Phase Deprotection of the Benzyl Moiety. − Synthesis of Compounds 7a−c, 10 and 13. − Typical Procedure: A catalytic amount of $Pd(OH)_2$/C (Pd 20%) was added to a stirred solution of **12** (23 mg, 0.05 mmol) in absolute EtOH (1 mL), and the slurry was vigorously stirred for 1 h at room temp. under hydrogen. $Pd(OH)_2$ was removed by filtration through a Celite pad, and the solvent was removed in vacuo to afford 14 mg of the pure compound **13**.

Compound 7a: Yield 88%, oil; R_f = 0.33 ($CHCl_3$/MeOH, 90:10). $[\alpha]_D^{23}$ = −21.3 (c = 0.9, MeOH). FTIR (KBr): ν_{max} = 3422, 1654, 1648, 1637, 1268 cm^{-1}. ^{1}H NMR (500 MHz, CD_3OD): δ = 4.40 (d, J = 5.5, 1 H), 3.60 (m, 1 H), 3.52 (q, J = 6.9, 1 H), 2.69 (dd, J= 15.6, 3.2, 1 H), 2.54 (dd, J = 15.6, 9.6, 1 H), 2.20 (m, 1 H), 1.28 (d, J = 6.9, 3 H), 1.01 (d, J = 7.3, 3 H), 0.99 (d, J = 7.3, 3 H). ^{19}F NMR (500 MHz, CD_3OD): δ = −76.2 (d, J = 6.8). ^{13}C NMR (125.6 MHz, CD_3OD): δ = 175.0, 173.7, 172.2, 128.1 (q, J = 285.8), 59.4, 56.1 (q, J = 28.0), 55.7, 36.1, 31.7, 20.2, 19.6, 18.4. MS (70 eV); *m*/*z* (%): 344 (58) [M^+ + 1], 212 (100).

Compound 7b: Yield 85%, oil; R_f = 0.39 (hexane/EtOAc, 50:50). $[\alpha]_D^{23}$ = −11.8 (c = 0.5, MeOH). FTIR (KBr): ν_{max} = 3415, 1653, 1632, 1262 cm^{-1}. ^{1}H NMR (500 MHz, CD_3OD): δ = 7.30−7.18 (m, 5 H), 4.59 (m,1 H), 3.50 (m, 1 H), 3.06 (m, 2 H), 3.00 (d, J = 6.4, 1 H), 2.59 (dd, J= 15.1, 4.6, 1 H), 2.44 (dd, J = 15.1, 8.7, 1 H), 1.85 (m, 1 H), 0.96 (d, J = 6.9, 3 H), 0.93 (d, J = 6.9, 3 H). ^{19}F NMR (500 MHz, CD_3OD): δ = −76.6 (d, J = 6.8). ^{13}C NMR (125.6 MHz, CD_3OD): δ = 172.4, 171.5, 138.2, 130.5, 129.4, 127.9 (q, J = 283.2), 127.8, 83.0, 66.4, 56.5 (q, J = 28.4), 56.2, 38.7, 36.4, 33.1, 28.2, 19.6, 18.9. MS (70 eV); *m*/*z* (%): 476 (57) [M^+ + 1], 415 (100).

Compound 7c: Yield 81%, oil; R_f = 0.31 (hexane/EtOAc, 50:50). $[\alpha]_D^{23}$ = + 51.9 (c = 0.9, MeOH). FTIR (KBr): ν_{max} = 3445, 1667, 1643 cm^{-1}. ^{1}H NMR (500 MHz, CD_3OD): δ = 4.42 (q, J = 7.3, 1 H), 3.72 (m, 3 H), 3.60 (m, 1 H), 3.33 (q, J = 6.9, 1 H), 2.61 (dd, J= 14.7, 3.3, 1 H), 2.44 (dd, J = 14.7, 8.7, 1 H), 1.40 (d, J = 7.3, 3 H), 1.25 (d, J = 6.9, 3 H). ^{19}F NMR (500 MHz, CD_3OD): δ = −77.6 (d, J = 7.5). ^{13}C NMR (125.6 MHz, CD_3OD): δ = 174.6, 173.8, 171.5, 127.5 (q, J = 281.8), 57.3 (q, J = 28.9), 56.2, 52.7, 49.6, 36.2, 19.9, 17.4.

Compound 10: Yield 88%, oil; R_f = 0.25 ($CHCl_3$/MeOH, 90:10). $[\alpha]_D^{23}$ = −59.5 (c = 0.5, MeOH). FTIR (KBr): ν_{max} = 1661, 1642, 1633, 1270 cm^{-1}. ^{1}H NMR (500 MHz, CD_3OD): δ = 7.31−7.16 (m, 5 H), 3.70 (m, 1 H), 3.56 (m, 1 H), 3.01 (dd, J = 13.3, 5.0, 1 H), 2.78 (dd, J = 13.3, 7.9, 1 H), 2.73 (s, 3 H), 2.45 (dd, J = 15.1, 3.2, 1 H), 2.20 (dd, J = 15.1, 10.1, 1 H). ^{19}F NMR (500 MHz, CD_3OD): δ = −76.2 (d, J = 6.7). ^{13}C NMR (125.6 MHz, CD_3OD): δ = 176.0, 168.8, 138.3, 130.3, 129.6, 127.9, 127.0 (q, J = 284.8), 56.1 (q, J = 28.0), 41.0, 33.9, 26.2. MS (70 eV); *m*/*z* (%): 334 (18) [M^+ + 1], 179 (100), 91 (25).

Compound 13: Yield > 98%; R_f = 0.11 ($CHCl_3$/MeOH, 90:10). $[\alpha]_D^{23}$ = −1.4 (c = 0.6, MeOH). ^{1}H NMR (500 MHz, CD_3OD): δ = 3.52 (m, 1 H), 3.22 (d, J = 5.0, 1 H), 2.49 (dd, J = 14.7, 5.0, 1 H), 2.35 (dd, J = 14.7, 7.8, 1 H), 1.96 (m, 1 H), 0.98 (d, J = 6.9, 3 H), 0.92 (d, J = 6.9, 3 H). ^{19}F NMR (500 MHz, CD_3OD): δ = −77.6 (d, J = 7.2). ^{13}C NMR (125.6 MHz, CD_3OD): δ = 178.1, 168.6, 127.8 (q, J = 282.0), 67.4, 57.5 (q, J = 28.5), 34.1, 33.0, 19.7, 18.2. MS (70 eV); *m*/*z* (%): 273 (16) [M^+ + 1], 257 (52), 227 (39), 211 (100), 152 (53).

Synthesis of Resin 15: A Erlenmeyer flask was charged with resin **14** (1.28 mmol/g, 1.3 equiv.), Fmoc-L-Ala-OH (3.9 g, 3.9 equiv.), HOBt (527 mg, 3.9 equiv.), DIC (0.611 mL, 3.9 equiv.), a catalytic amount of DMAP and a 9:1 mixture of CH_2Cl_2/DMF (20 mL). It was then shaken overnight at room temperature. The solution was drained and the resin was washed with DMF (3 × 20 mL), CH_2Cl_2 (3 × 20 mL), MeOH (3 × 20 mL), and CH_2Cl_2 (3 × 20 mL), and dried in vacuo to a constant weight.

Resin 15: FTIR (KBr): ν_{max} = 1671, 11652, 1611, 1384 cm^{-1}.

Solid-Phase Michael Addition. − Synthesis of Resin 17: A Erlenmeyer flask was charged with resin **15** (0.93 mmol/g, 1.3 equiv.)

and a 20% mixture of piperidine in dry DMF (16 mL), and then shaken for 1 h at room temperature. The solution was drained and the resin was washed with DMF (3 × 20 mL) and CH_2Cl_2 (5 × 20 mL), and dried in vacuo to a constant weight. The new resin was placed in a solid-phase reaction vessel with oxazolidin-2-one **16** (816 mg, 3.9 equiv.) and CH_2Cl_2 (24 mL), and then shaken for 3 d at room temperature. The solution was drained and the solvent was evaporated in vacuo to recover the unchanged compound **16** (544 mg, 2.6 equiv.). The resin was washed with CH_2Cl_2 (5 × 20 mL) and dried to a constant weight.

Resin 17: FTIR (KBr): ν_{max} = 1780, 1679, 1611, 1384 cm^{-1}.

Solid-Phase Cleavage of the Oxazolidin-2-one. − Synthesis of Resin 18: In a round-bottomed flask, the resin **17** (0.94 mmol/g, 1.2 equiv.) was suspended in a 4:1 mixture of THF/H_2O (23 mL). To this suspension were added, at 0 °C and under nitrogen, a 30% (in weight) aqueous solution of H_2O_2 (0.49 mL, 4.8 equiv.), followed by solid LiOH·H_2O (50 mg, 1.2 equiv.). The mixture was stirred for 2 h, the solution was then drained and the resin was washed with water (3 × 20 mL), MeOH (3 × 20 mL), and CH_2Cl_2 (3 × 20 mL), and dried to a constant weight.

Solid-Phase Coupling with α-Amino Esters. − Synthesis of Resins 20a−j, 25, 26 and 29. − Typical Procedure: A Erlenmeyer flask was charged with resin **18** (1.00 mmol/g, 0.10 equiv.), L-Gly-O*t*Bu·HCl (50 mg, 0.30 equiv.), HOAt (41 mg, 0.30 equiv.), DIC (0.047 mL, 0.3 equiv.), TMP (0.080 mL, 0.6 equiv.), a catalytic amount of DMAP, and dry DMF (2 mL). It was then shaken overnight at room temperature. The solution was drained and the resin was washed with DMF (3 × 20 mL), CH_2Cl_2 (3 × 20 mL), MeOH (3 × 20 mL), and CH_2Cl_2 (3 × 20 mL), and dried in vacuo to a constant weight.

Resin 20a: FTIR (KBr): ν_{max} = 1735, 1672, 1651, 1385 cm^{-1}.

Resin 20b: FTIR (KBr): ν_{max} = 1736, 1671, 1648, 1384 cm^{-1}.

Resin 20c: FTIR (KBr): ν_{max} = 1735, 1671, 1655, 1383 cm^{-1}.

Resin 20d: FTIR (KBr): ν_{max} = 1735, 1686, 1655, 1384 cm^{-1}.

Resin 20e: FTIR (KBr): ν_{max} = 1735, 1685, 1655, 1384 cm^{-1}.

Resin 20f: FTIR (KBr): ν_{max} = 1736, 1671, 1655, 1383 cm^{-1}.

Resin 20g: FTIR (KBr): ν_{max} = 1735, 1671, 1655, 1384 cm^{-1}.

Resin 20h: FTIR (KBr): ν_{max} = 1735, 1685, 1655, 1385 cm^{-1}.

Resin 20i: FTIR (KBr): ν_{max} = 1736, 1656 cm^{-1}.

Resin 20j: FTIR (KBr): ν_{max} = 1734, 1653 cm^{-1}.

Resin 25: FTIR (KBr): ν_{max} = 1734, 1647, 1384 cm^{-1}.

Resin 26: FTIR (KBr): ν_{max} = 1734, 1647, 1651, cm^{-1}.

Resin 29: FTIR (KBr): ν_{max} = 1655 cm^{-1}.

Solid-Phase Hydrolysis of the Ester Function. − Synthesis of Resins 22a−e. − Typical Procedure: In a round-bottomed flask, the resin **20e** (0.84 mmol/g, 0.10 equiv.) was suspended in a 4:1 mixture of THF/H_2O (2 mL). Solid LiOH·H_2O (17 mg, 0.4 equiv.) was added to this suspension at 0 °C. The mixture was stirred for 2 h, the solution was drained, and the resin was washed with water (3 × 20 mL), MeOH (3 × 20 mL), and CH_2Cl_2 (3 × 20 mL), and dried to a constant weight.

Resin 22a: FTIR (KBr): ν_{max} = 3398, 1608 cm^{-1}.

Resin 22c: FTIR (KBr): ν_{max} = 3436, 1639 (broad signal) cm^{-1}.

Resin 22d: FTIR (KBr): ν_{max} = 3423, 1638 (broad signal) cm^{-1}.

Resin 22e: FTIR (KBr): ν_{max} = 3433, 1636 (broad signal), 1441 cm^{-1}.

Cleavage from the Resin. − Synthesis of the Products 21a−j, 23a−e, 27, 28 and 30. − Typical Procedure: A Erlenmeyer flask was charged with resin **20i** (0.95 mmol/g, 0.10 equiv.) and 20% (in volume) TFA in CH_2Cl_2 (2 mL). It was then shaken for 1 h at room temperature. The solution was drained and the resin was washed three times with the same mixture. The solvent was evaporated in vacuo to recover 31 mg of the PM retro-ψ[NHCH(CF_3)]-peptidyl hydroxamate **21i**. The low stability of compounds **21a−h** precluded reliable characterization.

Compound 21i: Yield 60%, purity 87%. ^{1}H NMR (500 MHz, CD_3OD): δ = 3.95 (m, 4 H, -NHC*H*$_2$COOH, diast. a+b), 3.73 [m, 2 H, -C*H*(CH_3)-, diast. a + b], 3.64 [m, 1 H, -C*H*(CF_3)-, diast. a], 3.54 [m, 1 H, -C*H*(CF_3)-, diast. b], 2.66 [m, 2 H, -CH*H*CH(CF_3)-, diast. a + b], 2.50 [m, 2 H, -C*H*HCH(CF_3)-, diast. a + b], 1.30 [br. s, 6 H, -CH(C*H*$_3$)-, diast. a + b]. ^{19}F NMR (500 MHz, CD_3OD): δ = −76.5 (d, *J* = 5.0, 3 F, diast. a), −77.3 (br. s, 3 F, diast. b). CIMS; *m*/*z*: 302 $[M + 1]^+$.

Compound 21j: Yield 60%, purity 79%. ^{1}H NMR (500 MHz, CD_3OD): δ = 4.44 [q, *J* = 7.3 Hz, 1 H, -C*H*(CH_3)COOH, diast. a], 4.40 [q, *J* = 7.3, 1 H, -C*H*(CH_3)COOH, diast. b], 3.72 [m, 1 H, -C*H*(CH_3)-, diast. a], 3.61 [m, 1 H, -C*H*(CH_3)-, diast. b], 3.51 [m, 1 H, -C*H*(CF_3)-, diast. a], 3.41 [m, 1 H, -C*H*(CF_3)-, diast. b], 2.63 [m, 2 H, -CH*H*CH(CF_3)-, diast. a + b], 2.48 [m, 2 H, -C*H*HCH(CF_3)-, diast. a + b], 1.44 [d, *J* = 7.3, 3 H, -CH(C*H*$_3$)COOH, diast. a], 1.40 [d, *J* = 7.3, 3 H, -CH(C*H*$_3$)COOH, diast. b], 1.30 [br. s, 6 H, -CH(C*H*$_3$)-, diast. a + b]. ^{19}F NMR (500 MHz, CD_3OD): δ = −76.6 (d, *J* = 6.6, 3 F, diast. a), −77.4 (br. s, 3 F, diast. b). CIMS; *m*/*z*: 316 $[M + 1]^+$.

Compound 23a: Yield not determined, purity 73%. ^{1}H NMR (500 MHz, [D_6]DMSO): δ = 10.56 (br. s, 1 H, -N*H*OH, diast. a), 10.35 (br. s, 1 H, -N*H*OH, diast. b), 8.46 (br. s, 2 H, -CON*H*-, diast. a + b), 7.30−7.17 (m, 10 H, aromatics, diast a + b), 4.46 (m, 2 H, -C*H*CH_2Ph, diast. a + b), 3.65 [m, 2 H, -NHC*H*(CH_3)-, diast a + b], 3.50 [m, 2 H, -C*H*(CF_3)-, diast. a + b], 3.05 (m, 2 H, -CH*H*Ph, diast. a + b), 2.88 (m, 2 H, -C*H*HPh, diast. a + b), 2.50 [m, 2 H, -CH*H*CH(CF_3)-, diast. a + b], 2.41 [m, 2 H, -C*H*HCH(CF_3)-, diast. a + b], 1.13 (d, *J* = 5.9, 3 H, -CHC*H*$_3$, diast a), 1.11 (d, *J* = 6.1, 3 H, -CHC*H*$_3$, diast b). ^{19}F NMR (500 MHz, [D_6]DMSO): δ = −74.0 (d, *J* = 7.1, 3 F, diast. a), − 74.9 (d, *J* = 6.0, 3 F, diast. b). CIMS; *m*/*z*: 391 $[M + 1]^+$.

Compound 23d: Yield > 98%, purity 96%. ^{1}H NMR (500 MHz, CD_3OD): δ = 4.50 [t, *J* = 7.3, 1 H, -C*H*(*i*Bu), diast. a], 4.45 [dd, *J* = 9.6, 5.5, 1 H, -C*H*(*i*Bu), diast. b], 3.65 [m, 4 H, -C*H*(CH_3)- + −C*H*(CF_3)-, diast. a + b], 2.66 [m, 2 H, -CH*H*CH(CF_3)-, diast. a + b], 2.51 [m, 2 H, -C*H*HCH(CF_3)-, diast. a + b], 1.73 [m, 2 H, -C*H*(CH_3)$_2$, diast. a + b], 1.65 [m, 4 H, -CH_2CH(CH_3)$_2$, diast. a + b], 1.30 [m, 6 H, −CH(C*H*$_3$)-, diast. a + b], 0.98 [d, *J* = 6.9, 3 H, -(CH_3)CH(C*H*$_3$)-, diast a], 0.97 [d, *J* = 6.1, 3 H, -(CH_3)CH(C*H*$_3$)-, diast b], 0.95 [d, *J* = 6.9, 3 H, -(C*H*$_3$)CH(CH_3)-, diast a], 0.93 [d, *J* = 6.1, 3 H, -(C*H*$_3$)CH(CH_3)-, diast b]. ^{19}F NMR (500 MHz, CD_3OD): δ = −76.0 (d, *J* = 6.0, 3 F, diast. a), −77.3 (br. s, 3 F, diast. b). CIMS; *m*/*z*: 358 $[M + 1]^+$.

Compound 23e: Yield 80%, purity 74%. ^{1}H NMR (500 MHz, CD_3OD): δ = 4.52 [dd, *J* = 8.7, 2.7, 1 H, -C*H*(COOH)-, diast. a], 4.46 [dd, *J* = 7.8, 2.0, 1 H, -C*H*(COOH)-, diast. b], 3.67 [m, 8 H, −C*H*(CH_3)- + -C*H*(CF_3)- + -C*H*$_2$N(CO)-, diast. a + b], 2.70 [m, 4 H, -CH*H*CH(CF_3)-, diast. a + b], 2.28 [m, 1 H, -C*H*HCH(COOH)-, diast. a + b], 2.05 [m, 3 H, -CH*H*CH(COOH)- + -C*H*$_2$CH$_2$N(CO-)-, diast. a + b], 1.30 [br. s, 6 H, -CH(C*H*$_3$)-, diast. a + b]. ^{19}F NMR (500 MHz, CD_3OD): δ = −76.3 (br. s, 3 F, diast. a), −77.1 (br. s, 3 F, diast. b). CIMS; *m*/*z*: 341 $[M]^+$.

Compound 27: Yield 68%, purity > 98%. ^{1}H NMR (500 MHz, CD_3OD): δ = 7.30−7.18 (m, 10 H, aromatics, diast. a + b), 4.66 [m, 2 H, −C*H*(COOH)-, diast. a + b], 4.46 [dd, *J* = 8.7, 5.9, 1 H, -C*H*(*i*Bu)-, diast. a], 4.39 [t, *J* = 7.8, -C*H*(*i*Bu)-, diast. b], 3.71 [m, 1 H, -C*H*(CH_3)-, diast. a], 3.61 [m, 1 H, −C*H*(CH_3)-, diast. b], 3.57 [m, 1 H, -C*H*(CF_3)-, diast. a], 3.42 [m, 1 H, -C*H*(CF_3)-, diast. b], 3.20 [m, 2 H, -CH*H*(Ph)-, diast. a + b], 3.02 [m, 2 H, -C*H*H(Ph)-, diast. a + b], 2.60 [m, 2 H, -CH*H*CH(CF_3)-, diast. a + b], 2.45 [m, 2 H, -C*H*HCH(CF_3)-, diast. a + b], 1.65 [m, 2 H, -C*H*(CH_3)$_2$, diast. a + b], 1.53 [m, 4 H, −C*H*$_2$CH(CH_3)$_2$, diast. a + b], 1.29 [d, *J* = 6.4, 3 H, -CH(C*H*$_3$)-, diast. a], 1.26 [d, *J* = 6.4, 3 H, -CH(C*H*$_3$)-, diast. b], 0.94 [d, *J* = 6.4, -(CH_3)CH(C*H*$_3$), diast. a], 0.93 [d, *J* = 6.4, -(C*H*$_3$)CH(CH_3), diast. a], 0.91 [d, *J* = 7.3, -(CH_3)CH(C*H*$_3$), diast. b], 0.89 [d, *J* = 7.3, -(C*H*$_3$)CH(CH_3), diast. b]. ^{19}F NMR (500 MHz, CD_3OD): δ = −76.1 (d, *J* = 6.3, 3 F, diast. a), − 77.0 (d, *J* = 6.3, 3 F, diast. b). CIMS; *m*/*z*: 504 $[M]^+$.

Compound 28: Yield 95%, purity 92%. ^{1}H NMR (500 MHz, CD_3OD): δ = 4.52 [q, *J* = 7.3, 1 H, -CONHC*H*(CH_3)-, diast. a], 4.50 [q, *J* = 6.9, 1 H, -CONHC*H*(CH_3)-, diast. b], 4.38 [d, *J* = 5.5, 1 H, -C*H*(*i*Pr)-, diast. a], 4.33 [d, *J* = 5.5, 1 H, -C*H*(*i*Pr)-, diast. b], 3.78 [m, 1 H, HONHNCOC*H*(CH_3)-, diast a], 3.66 [m, 1 H, HONHNCOC*H*(CH_3)-, diast. b], 2.67 [m, 2 H, -CH*H*CH(CF_3)-, diast. a + b], 2.51 [m, 2 H, -C*H*HCH(CF_3)-, diast. a + b], 2.18 [m, 2 H, -C*H*(CH_3)$_2$, diast. a + b], 1.38 [m, 6 H, -CH(C*H*$_3$)-, diast. a + b], 1.31 [m, 6 H, -CH(C*H*$_3$)-, diast. a + b], 0.96 [m, 12 H, −CH(C*H*$_3$)$_2$, diast. a + b]. ^{19}F NMR (500 MHz, CD_3OD): δ = −76.2 (d, *J* = 6.8, 3 F, diast. a), − 76.9 (m, 3 F, diast. b). CIMS; *m*/*z*: 414 $[M]^+$.

Compound 30: Yield not determined, purity 73%. ^{1}H NMR (500 MHz, [D$_6$]acetone): δ = 7.30−7.16 (m, 10 H, aromatics, diast. a + b), 4.70 [m, 1 H, -C*H*(CH_2Ph)-, diast. a], 4.63 [m, 1 H, -C*H*(CH_2Ph)-, diast. b], 3.61 [m, 4 H, -C*H*(CH_3)- + C*H*(CF_3)-, diast. a + b], 3.14 (m, 2 H, -CH*H*Ph, diast. a + b), 2.95 [m, 2 H, -C*H*H(Ph), diast. a + b], 2.69 (m, 6 H, -NHC*H*$_3$, diast. a + b), 2.53 [m, 2 H, -CH*H*CH(CF_3)-, diast. a + b], 2.45 [m, 2 H, -C*H*HCH(CF_3)-, diast. a + b], 1.27 [m, 6 H, -CH(C*H*$_3$)-, diast. a + b]. ^{19}F NMR (500 MHz, [D$_6$]acetone): δ = −75.7 (d, *J* = 4.1, 3 F, diast. a), − 76.6 (m, 3 F, diast. b). CIMS; *m*/*z*: 405 $[M + 1]^+$.

Tests on MMP-9. − Cell Culture: Circulating human monocytes were isolated from blood of healthy donors as previously described.[20] The monocytes were collected, washed, resuspended in serum-free Dulbecco Modified Eagle's medium (GIBCO BRL, Life Technologies, Italia) and plated at a density of 3×10^6 cells in a 35-mm dish. After 2 h, cell monolayers were washed twice and the adherent cells were incubated for 10−14 d with DMEM containing 10% human AB serum and insulin 8 μg/mL, to allow for differentiation in macrophages. To generate the conditioned media, cells were incubated for 24 h at 37 °C with DMEM, supplemented with 0.2% bovine serum albumin (BSA; Sigma) and the indicated concentrations of compounds. At the end of the incubation, the conditioned media were collected and the gelatinolytic capacity of secreted MMP-9 analysed by zymography.[20] Cellular protein content was measured according to Lowry.[21]

SDS Page Zymography: Samples (5 μL of conditioned medium per lane) were subjected to electrophoresis at 4 °C on 7.5% polyacrylamide gels containing 10% SDS and gelatin (1 mg/mL) under nonreducing conditions and without boiling. After electrophoresis, SDS was removed from gels in two washes with 2.5% Triton X-100 (Sigma) at room temperature. After the washes, the gels were incubated overnight at 37 °C with gentle shaking in TRIS (50 mM, pH = 7.5) containing NaCl (150 mM), $CaCl_2$ (10 mM), and $ZnCl_2$ (1 μM), to activate the ability of the metalloproteinase to digest the substrate. For inhibition studies and to confirm the identity of MMP-9, identical gels were incubated in the above buffer containing either EDTA (20 mM), an inhibitor of MMPs, or PMSF (1 mM), an inhibitor of serine proteases. The addition of PMSF did not alter the MMP-9 gelatinolytic capacity, while the treatment with EDTA completely abolished it (data not shown). At the end of the incubation, the gels were stained with a solution of 0.1% Coomassie brilliant blue R-250 (Sigma) in 25% methanol and 7% acetic acid. Clear zones against the blue background indicated the presence of proteinolytic activity.[22]

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[22] Abbreviations: EDTA, ethylenediaminetetraacetic acid; DMEM, Dulbecco Modified Eagle's medium; BSA, bovine serum albumin; PMSF, phenylmethylsulfonyl fluoride; SDS, sodium dodecyl sulfate.

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