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# Toward a fragment-based approach to MMPs inhibitors: an expedite and efficient synthesis of *N*-hydroxylactams

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ABSTRACT

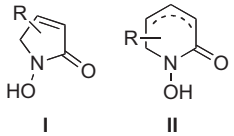
Matrix metalloproteinases (MMPs), a class of zinc-enzymes over-activated in many pathologies, such as arthritis and cancer, can be efficiently inhibited by a variety of molecules bearing zinc-binding groups (ZBGs). The hydroxamic acid moiety represents one of the most potent and widely exploited ZBG but the poor target selectivity and in vivo toxicity have tempered the initial enthusiasm for this class of potential therapeutics. These drawbacks might be circumvented, at least in part, by increasing the structural constraints around the hydroxamic moiety. Following this strategy we designed and prepared *N*-hydroxylactam molecules of different size through a synthetic protocol based on a ring closing metathesis amenable to a fragment-based approach potentially leading to a large molecular diversity.



Metal ions play a relevant role in several physiological and pathological processes,[1] being involved, chiefly as enzyme cofactors,[2] in a variety of biochemical transformations[3] including redox reactions triggering oxidative stress in many neurological inflammatory disorders.[4] An increased level of copper and iron in well localized tissues and cellular brain districts has been observed in Parkinson's and Alzheimer's diseases, two widespread neurological disorders characterized by a metal-catalyzed formation of toxic radical species and metal-promoted aggregation of amyloid peptides[5] and alpha-synuclein.[6]

Matrix metalloproteinases (MMPs), are zinc-containing proteolytic enzymes with potent zinc-mediated endopeptidase activity against several protein components of both extracellular matrix and basal membrane.[7] The activity of MMPs is fundamental for maintaining a number of key physiologic processes such as tissue turn-over and embryonic development. In addition, over-expression of MMPs is responsible for many pathologies such as arthritis and cancer.[3,8] For this reason, great efforts have been made to set molecular chemical strategies for an effective and stable coordination of zinc ion to inhibit specific MMPs and possibly block cancer and associated metastatic processes triggered by their over-activation.[9,10] In view of this, the identification of suitable scaffold molecules bearing zinc-binding groups (ZBGs), such as hydroxamic acid and thiol units, is a well established approach for the development of potent MMPs and histone deacetylase (HDACs) inhibitors.[11,12] Unfortunately, the lack of target selectivity of many hydroxamic acid derivatives along with their pronounced toxicity has to some extent discouraged further clinical developments of this class of compounds. Since these negative features might also be associated with a high conformational mobility of most of the explored hydroxamic acid derivatives, we planned the synthesis of a series of molecules containing the hydroxamic acid moiety constrained in a cycle. The general structures **I** and **II** of the designed rigid molecules are illustrated in Chart 1. Five- and six-membered *N*-hydroxylactams were exploratively synthesized. Structures **II** can allocate a double bond either in the α,β- or in the β,γ-position.

The introduction of a strong structural constrain as a cyclic moiety, was pursued to bias molecular selectivity, which is still the main drawback of the majority of the already published linear congeners carrying the hydroxamic acid moiety. On the other hand, the intentionally reduced conformational flexibility, in addition to a suitable substitution pattern 'clock' around the *N*-hydroxylactam scaffold was instead conceived to enable the selective inhibition




I II

**Chart 1.** General structures of the targeted *N*-hydroxylactams.

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of MMPs as well as to control the toxicity profile often associated with more flexible MMP inhibitors. Furthermore, as showed in Chart 2, the proposed chemical pathway might enable the design of focused libraries of potential MMPs inhibitors through a fragment-based approach.[13]

The general retrosynthetic protocol envisioned for the synthesis of compounds with general structures **I** and **II** is depicted in Chart 2, where PG stands for protecting group. It is based on the coupling of differently substituted olefins with a suitable O-protected hydroxylamine, such as *O*-(*p*-methoxybenzyl)hydroxylamine (PMB-$ONH_2$), followed by a final ring closing metathesis (RCM) reaction catalyzed by the second generation Grubbs catalyst.[14] Although the synthetic strategy showed in Chart 2 could be in principle applied to the preparation of variously sized cycles, in this Letter we focused our attention on the synthesis of compounds bearing only five- and six-membered *N*-hydroxylactam rings. The synthesis of these derivatives is summarized in Schemes 1–4.

The preparation of five-membered ring derivatives (Scheme 1) was accomplished in three chemical steps starting from PMB-$ONH_2$ (**1**), which was reacted with acryloyl chloride in dichloromethane (DCM), using triethylamine (TEA) as a base.

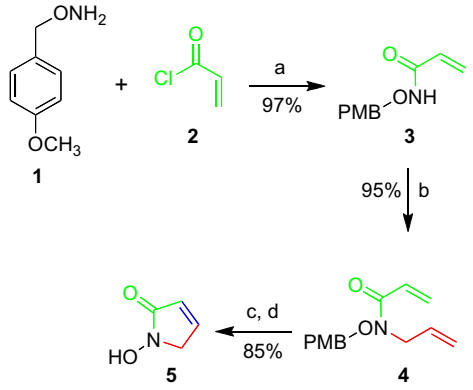
Under these experimental conditions, intermediate **3** was obtained in 97% yield. This intermediate was alkylated with allyl bromide in $CH_3CN$ using $K_2CO_3$ as a base to afford **4**, in 95% yield, which was then cyclized using the second generation Grubbs catalyst under RCM conditions to give after TFA deprotection the *N*-hydroxy-γ-lactam **5** in 85% yield over two steps. The synthesis of compounds of general structure **II** was carried out following two different approaches based on the position of the endocyclic double bond.

The synthesis of β,γ-unsaturated *N*-hydroxy-δ-lactam **9** (Scheme 2) was performed starting from **1**, which was coupled with but-3-enoic acid using diisopropylcarbodiimmide (DIC) and *N*-hydroxysuccinimide (NHS) in dry DCM.

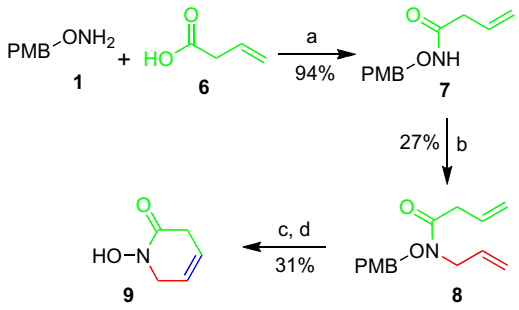
Under these experimental conditions, compound **7** was obtained in 94% yield. The subsequent alkylation of **7** under the same reaction conditions reported in Scheme 1 gave rise to a remarkable reduction of yield (27% vs 95%) due to the isomerization of terminal alkene double bond. Key intermediate **8** was cyclized under RCM conditions and then deprotected by TFA affording the final product **9** in 31% yield over two steps. Unlike compound **9**, the α,β-unsaturated isomer was prepared following a diverse chemical approach (Scheme 3).

The reaction of intermediate **3** with 1-(1-bromobut-3-en-1-yl)-4-nitrobenzene **10** under SN conditions resulted in a disappointing 9% yield. The subsequent RCM followed by TFA deprotection of the 4-nitrophenyl derivative **12** gave the expected *N*-hydroxylactam **13** in good yield (97% over the two steps).[15] In order to improve the yield of the critical SN alkylation reaction, we used an $Ag_2O$-based coupling reaction[16] (Scheme 4), resulting in a significant increase of yields (63% vs 9%).[17]

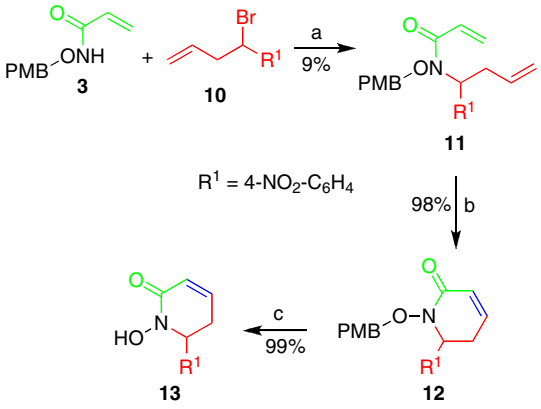
The synthetic approach proposed herein allowed the facile and versatile construction of *N*-hydroxy-γ and δ-lactam derivatives. Moreover, our reaction pathway may enable the introduction of a range of chemically diverse substituents on the carbon directly bound to the nitrogen atom of the hydroxamate unit as depicted in Chart 2. Consequently, the potential for a fragment-based approach for the design and synthesis of libraries of MMP inhibitors appears clear and feasible. As discussed above, the key step of our synthetic pathway is the RCM reaction of the diene intermediates to obtain the corresponding cyclic *N*-hydroxylactams. The only concern of our synthetic approach is the alkylation reaction because of the occurrence of competitive side reactions. One of these is the isomerization of the terminal double bond, as for compound **8** or the HBr elimination for reagent **10**, used in the synthesis of the α,β-unsaturated six-membered ring in **13**. To prevent the latter side reaction, we utilized an alternative chemical approach based



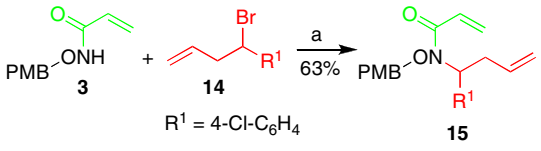

**Scheme 1.** Synthesis of five-membered ring derivative **5**. Reagents and conditions: (a) TEA, DCM, 0 °C; (b) allyl bromide, $K_2CO_3$, $CH_3CN$, reflux, (c) Grubbs II catalyst, toluene, 60 °C, 1 h; (d) TFA, triethylsilane, DCM, 0 °C.




**Scheme 2.** Synthesis of six-membered ring β,γ-unsaturated derivative **9**. Reagents and conditions: (a) DIC, NHS, dry DCM, room temperature; (b) allyl bromide, $K_2CO_3$, $CH_3CN$, reflux; (c) Grubbs II, toluene, 60 °C, 1 h; (d) TFA, triethylsilane, DCM, 0 °C.




**Scheme 3.** Synthesis of six-membered ring α,β-unsaturated derivative **13**. Reagents and conditions: (a) 1-(1-bromobut-3-en-1-yl)-4-nitrobenzene, $K_2CO_3$, $CH_3CN$, reflux; (b) Grubbs II catalyst, toluene, 60 °C, 1 h; (c) TFA, triethylsilane, DCM, 0 °C.




**Scheme 4.** $Ag_2O$-mediated coupling. Reagents and conditions: $Ag_2O$, $MgSO_4$, $Et_2O$, room temperature.

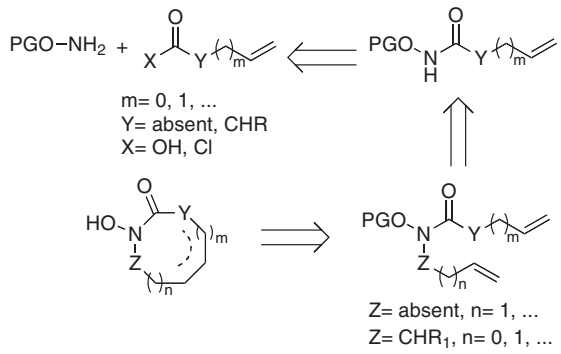



**Chart 2.** Retrosynthetic scheme.

on the use of $Ag_2O$ to perform the coupling between the O-protected hydroxamic acid intermediate and the suitable bromoalkyl derivatives. The use of such reaction conditions afforded a significant improvement of the yield.

The versatility of the present approach is witnessed also by the chance of obtaining six- and theoretically highly membered rings bearing the endocyclic double bound at diverse positions. It goes without saying that the double bond may be conveniently reduced to obtain the corresponding fully saturated derivatives.

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15. Synthesis of compound **13**
To a solution of **1** (6 mmol, 0.92 g) in $CH_2Cl_2$ (40 mL) and $Et_3N$ (10 mmol, 1.4 mL), cooled at 0 °C by an external ice-bath, acryloyl chloride (2 mmol, 0.16 mL) was added dropwise over ten minutes. The reaction mixture was stirred at 0 °C for 15 min, then extracted with a 2 N solution of HCl (3 × 10 mL), washed with brine (3 × 10 mL), dried over anhydrous $Na_2SO_4$ and finally concentrated to dryness under vacuum. The oily residue was purified by chromatography on silica gel using hexane/EtOAC 85/15 as the eluent affording 0.40 g (97%) of pure **3**. The latter (1.9 mmol, 0.39 g) was dissolved in anhydrous $CH_3CN$ (20 mL) and reacted with **10** (2.1 mmol, 0.53 g) for 4 h at reflux temperature under magnetic stirring using $K_2CO_3$ (2.6 mmol, 0.42 g) as a base. The inorganic residue was filtered-off and the filtrate concentrated under reduced pressure and purified by chromatography on silica gel using hexane/EtOAC 90/10 as eluent to afford 65 mg (9%) of **11** as a light brown oil.
*N-[(4-methoxybenzyl)oxy]-N-[1-(4-nitrophenyl)but-3-enyl]acrylamide* ***11***
$^1$H NMR ($CDCl_3$) $\delta$: 8.04 (d, 2H, $J$ = 8.8 Hz); 7.27 (d, 2H, $J$ = 8.8 Hz); 7.14 (d, 2H, $J$ = 8.8 Hz); 6.83 (d, 2H, $J$ = 8.8 Hz); 6.07 (dd, 1H, $J$ = 17.3, 10.7 Hz); 5.85 (dd, 1H, $J$ = 17.3, 1.6 Hz); 5.75 (t, 1H, $J$ = 6.6 Hz); 5.71–5.57 (m, 1H); 5.41 (dd, 1H, $J$ = 10.4, 1.6 Hz); 5.04–4.96 (m, 2H); 4.86–4.73 (m, 2H); 3.82 (s, 3H); 2.74–2.64 (m, 1H); 2.56–2.46 (m, 1H). ESI-MS for $C_{21}H_{22}N_2O_5$: $m/z$ $[M+Na]^+$ = 405.
Sixty milligrams (0.16 mmol) of **11** were dissolved in toluene (2.5 mL) and treated with II generation Grubbs catalyst (32 mg) at 60 °C under magnetic stirring for 20 min. The mixture was concentrated under reduced pressure then purified on silica gel using hexane/EtOAC 80/20 as the eluent to give 55 mg (98%) of **12**. The latter was dissolved in $CH_2Cl_2$ (0.5 mL) and treated with TFA (0.05 mL) and triethylsilane (0.005 mL) under magnetic stirring for 20 min. The solvent was removed under reduced pressure and the oily residue was treated with a few drops of $Et_2O$ affording the final product **13** in quantitative yield as a pale yellow solid.
*1-Hydroxy-6-(4-nitrophenyl)-5,6-dihydropyridin-2(1H)-one* ***13***
Mp 66–68 °C. $^1$H NMR ($CDCl_3$) $\delta$: 8.27 (d, 2H, $J$ = 8.6 Hz); 7.62 (d, 2H, $J$ = 8.6 Hz); 7.10 (br s, 1H); 6.30–6.40 (m, 1H); 6.20–6.14 (m, 1H); 5.30 (dd, 1H, $J$ = 9.9, 4.4 Hz); 2.70–2.50 (m, 2H). ESI-MS for $C_{11}H_{10}N_2O_4$: $m/z$ $[M-H]^-$ = 233.
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17. $Ag_2O$-mediated coupling reaction: preparation of compound **15**
To a solution of 3 (1.9 mmol, 0.39 g) in $Et_2O$ (20 mL) were added **14** (6.4 mmol, 1.6 g), $Ag_2O$ (1.9 mmol, 1.4 g), and $MgSO_4$ (40 mg). After stirring at room temperature for 12 h, the mixture was concentrated under reduced pressure and then purified on silica gel using hexane/EtOAC 80/20 as the eluent to afford 0.47 g (63%) of **15** as light brown oil.
*N-[1-(4-Chlorophenyl)but-3-enyl]-N-[(4-methoxybenzyl)oxy]acrylamide* ***15***
$^1$H NMR ($CDCl_3$) $\delta$: 7.26 (d, 2H, $J$ = 8.5 Hz); 7.19 (d, 2H, $J$ = 8.5 Hz); 7.06 (d, 2H, $J$ = 8.5 Hz); 6.77 (d, 2H, $J$ = 8.5 Hz); 6.67 (dd, 1H, $J$ = 17.3, 11.0 Hz); 5.96 (dd, 1H, $J$ = 17.3, 1.9 Hz); 5.78–5.64 (m, 1H); 5.53–5.45 (m, 2H); 5.07–5.03 (m, 1H); 5.01 (t, 1H, $J$ = 1.1 Hz); 4.60–4.80 (m, 2H); 3.80 (s, 3H); 2.71–2.60 (m, 1H); 2.55–2.46 (m, 1H). ESI-MS for $C_{21}H_{22}ClNO_3$: $m/z$ $[M+Na]^+$ = 394.