

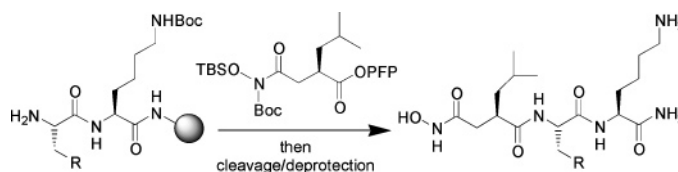
Solid-Phase Synthesis of Succinylhydroxamate Peptides: Functionalized Matrix Metalloproteinase Inhibitors

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ABSTRACT



A novel solid-phase synthesis strategy toward succinylhydroxamate peptides, using an appropriately protected hydroxamate building block, is described. Rapid and efficient access is gained to amine-functionalized peptides, which can be decorated with, for instance, a fluorescent label. In addition, we demonstrate an on-resin synthesis of a biotinylated photoactivatable hydroxamate peptide, which can be used as an activity-based probe for matrix metalloproteinases and ADAMs.

Matrix metalloproteinases (MMPs) play a central role in the degradation of extracellular matrix proteins (gelatin, elastin, and collagen). Disturbances in their physiological regulation can contribute to a wide range of pathological states, such as tumor invasion, atherosclerosis, and arthritis.¹ ADAMs are membrane-bound proteins containing both a cellular adhesion and an MMP domain² and are for instance involved in cell migration, muscle development, and fertilization. Some ADAMs display sheddase activity,³ as exemplified by ADAM-17 (also known as the TNF α converting enzyme or TACE). The biological importance of these two classes of enzymes, together with the fact that their activities are highly regulated in vivo at both the gene expression and protein levels, has

made the development of chemical tools for their study (inhibitors and activity-based probes⁴) an important and active field of research in recent years.

The most commonly used MMP and ADAM inhibitors fall into the class of succinylhydroxamate-containing peptide analogues.⁵ Notably, commercially available members of this type are marimastat, batimastat, and TAPI-2 (see Figure 1), each

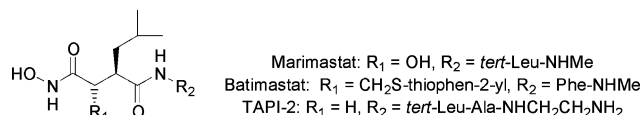


Figure 1. Structures of some commercially available MMP/ADAM inhibitors.

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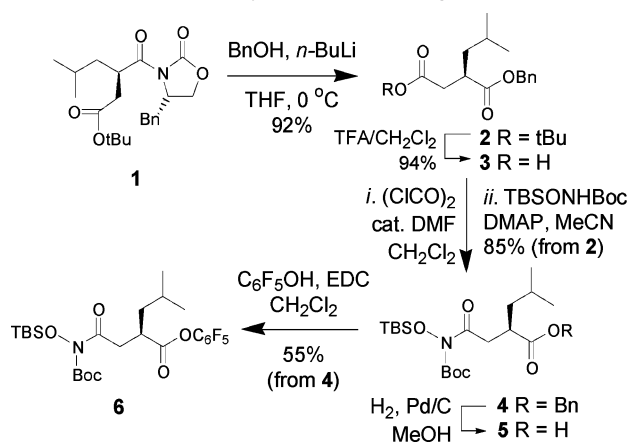
(1) Johnson, L. J.; Dyer, R.; Hupe, D. J. *Curr. Opin. Chem. Biol.* **1998**, 2, 466–471.

(2) (a) Seals, D.; Courtneidge, S. A. *Genes Dev.* **2003**, 17, 7–30. (b) Black, R. A.; White, J. M. *Curr. Opin. Cell Biol.* **1998**, 10, 654–659.

displaying sub- to low-nanomolar, broad MMP/ADAM inhibitory activity.

Because these compounds are peptide-like structures, it would be highly convenient to be able to synthesize functionalized derivatives using standard solid-phase peptide synthesis (SPPS) procedures. Although many SPPS procedures for C-terminal hydroxamic acids have been reported,⁶ there are very few SPPS methods for N-terminal succinyl-hydroxamate peptides^{7,8} which obviate the handling of advanced hydroxamate precursors such as carboxylic acids⁹ or esters.¹⁰ Therefore, we devised a building block that can be used in a linear SPPS strategy, immediately leading to products with R₁ = H. In this paper, we describe the novel building block **6** (see Scheme 1) and its use in the synthesis

Scheme 1. Synthesis of Building Block **6**



of inhibitors and functionalized probes for the study of MMP and ADAM activities.

(3) Huovila, A.-P. J.; Turner, A. J.; Peltö-Huikko, M.; Kärkkäinen, I.; Ortiz, R. M. *Trends Biochem. Sci.* **2005**, *30*, 413–422.

(4) For a recent overview, see: Verhelst, S. H. L.; Bogoy, M. *QSAR Comb. Sci.* **2005**, *24*, 261–269.

(5) Whittaker, M.; Floyd, C. D.; Brown, P.; Gearing, A. J. H. *Chem. Rev.* **1999**, *99*, 2735–2776.

(6) See, for instance: (a) Mellor, S. L.; McGuire, C.; Chan, W. C. *Tetrahedron Lett.* **1997**, *38*, 3311–3314. (b) Sasubilli, R.; Gutheil, W. G. *J. Comb. Chem.* **2004**, *6*, 911–915. (c) Gazal, S.; Masterson, L. R.; Barany, G. *J. Pept. Res.* **2005**, *66*, 324–332. (d) Yin, Z.; Low, K. S.; Lye, P. L. *Synth. Commun.* **2005**, *35*, 2945–2950.

(7) Solid-phase strategies of marimastat analogues have been reported: (a) Jenssen, K.; Sewald, K.; Sewald, N. *Bioconjugate Chem.* **2004**, *15*, 594–600. (b) Barlaam, B.; Koza, P.; Berriot, J. *Tetrahedron* **1999**, *55*, 7221–7232.

(8) During the preparation of this manuscript, a paper appeared reporting the synthesis of a library of diastereomeric inhibitors with R = H by using an alternative, racemic building block with *O*-trityl protection: Wang, J.; Uttamchandani, M.; Sun, L. P.; Yao, S. Q. *Chem. Commun.* **2006**, 717–719.

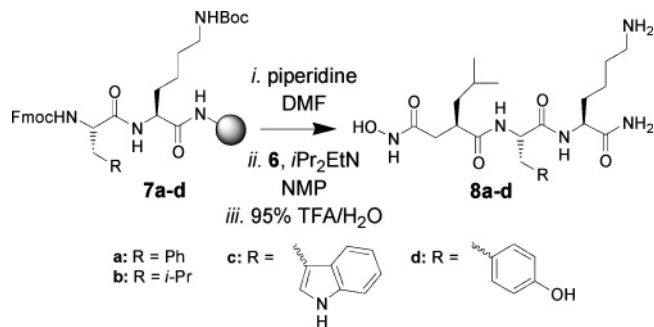
(9) For selected examples of hydroxamate solution syntheses from carboxylic acids, see: (a) Reddy, A. S.; Kumar, M. S.; Reddy, G. R. *Tetrahedron Lett.* **2000**, *41*, 6285–6288. (b) Giacomelli, G.; Porcheddu, A.; Salaris, M. *Org. Lett.* **2003**, *5*, 2715–2717.

(10) For selected examples of hydroxamate solution syntheses from esters, see: (a) Levy, D. E.; Lapierre, F.; Liang, W.; Ye, W.; Lange, C. W.; Li, X.; Grobelny, D.; Casabonne, M.; Tyrrell, D.; Holme, K.; Nadzan, A.; Galardy, R. E. *J. Med. Chem.* **1998**, *41*, 199–223. (b) Ho, C. Y.; Strobel, E.; Ralbovsky, J.; Gallemmo, R. A., Jr. *J. Org. Chem.* **2005**, *70*, 4873–4875.

The synthesis of **6** starts with the known compound **1**.^{10a,11} Removal of the chiral auxiliary using lithium benzyl alcoholate gave benzyl ester **2**. Partial deprotection led to monoester **3**, the acyl chloride derivative of which was reacted with *N*-Boc-*O*-TBS-hydroxylamine.¹² Next, the benzyl ester was removed by catalytic hydrogenation to obtain free acid **5**. It was soon discovered that **5** is not only labile during storage (even at –20 °C) but also extremely base sensitive. Attempts to precipitate it as several different alkylammonium salts led to complete degradation. Also, standard peptide coupling conditions (HCTU/*i*Pr₂EtN) led to complicated reaction mixtures, presumably due to cyclization of **5** to the anhydride. Therefore, to minimize the amount of base encountered by compound **5**, it was decided to prepare an active ester derivative, which can, in theory, be coupled without additional base. The pentafluorophenyl (PFP) ester **6**, obtained by reaction of **5** with pentafluorophenol under the influence of *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide (EDC), proved to be far more stable during storage than acid **5**.

Next, the potential of PFP ester **6** in SPPS was evaluated (see Scheme 2). Dipeptide **7a** was synthesized on Rink amide

Scheme 2. Solid-Phase Synthesis of Succinylhydroxamate Peptides Using **6**



resin using standard protocols. After removal of the Fmoc group, several conditions for the coupling of **6** were investigated.¹³ The optimal conditions proved to be shaking the resin for 2 h with 5 equiv of **6** and 2 equiv of *i*Pr₂EtN relative to the resin-bound peptide in NMP. The resulting product was cleaved from the resin and concomitantly deprotected using 95% aqueous TFA, cleanly yielding peptide **8a** in 64% yield after HPLC purification. In the same fashion, the analogous peptides in which phenylalanine is replaced by leucine (**8b**, 56% yield), tryptophan (**8c**, 42% yield), and tyrosine (**8d**, 71% yield) were synthesized.

Enzyme inhibition tests using a fluorogenic substrate (see Supporting Information) revealed IC₅₀ values in the low

(11) Augé, F.; Hornebeck, W.; Decarme, M.; Laronze, J.-Y. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1783–1786.

(12) Altenburger, J. M.; Mioskowski, C.; d'Orchymont, H.; Schirlin, D.; Schalk, C.; Tarnus, C. *Tetrahedron Lett.* **1992**, *33*, 5055–5058. Instead of Et₃N and DMAP for the coupling of the hydroxylamine to the acyl chloride, we used 2 equiv of DMAP without Et₃N because the acyl chloride of **3** appeared to be highly sensitive to tertiary amine bases.

(13) Five equivalents of **6** and no additional base gave incomplete, though clean, coupling, and adding five equivalents of *i*Pr₂EtN resulted in complete consumption of **7a** but also substantial side reactions.

nanomolar range for the four compounds against both MMP-12 (catalytic domain) and ADAM-17 (ectodomain), as shown in Table 1, entries 1–4. The observation that the aromatic

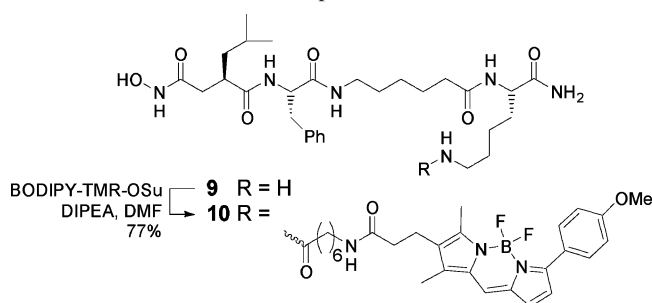
Table 1. Inhibitory Activities of Newly Synthesized Compounds (IC₅₀ Values in nM)

entry	compound	MMP-12	ADAM-17
1	8a	5.3	15.1
2	8b	41.8	58.2
3	8c	7.8	26.1
4	8d	4.8	10.7
5	9	4.1	23.6
6	10	13.1	42.7
7	11	3.6	20.6

amino-acid-containing compounds **8a,c,d** are more potent inhibitors than the aliphatic **8b** corroborates earlier findings.⁵

Functionalization of the free amine in the prepared inhibitors with a fluorescent label can readily be accomplished, as demonstrated in Scheme 3. Compound **9**, a

Scheme 3. Fluorescent Labeling of a Succinylhydroxamate Peptide

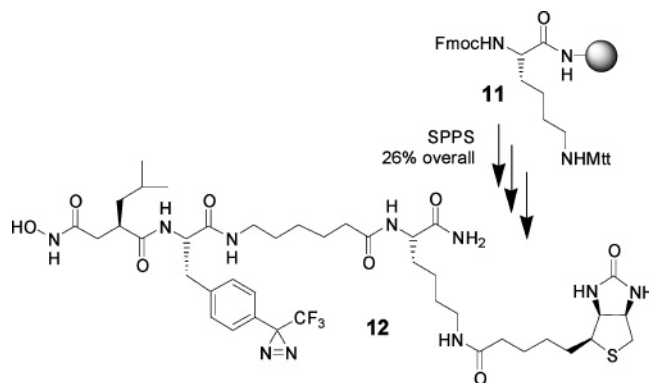


modified version of **8a** with an additional spacer, was obtained via the presented method in 53% overall isolated yield. It was established that this spacer does not significantly influence the inhibitory activity (see entry 5, Table 1). Reaction of **9** in DMF in the presence of 1 equiv of BODIPY-TMR-OSu and *i*Pr₂EtN cleanly furnished labeled compound **10** in 77% isolated yield. Incorporation of the fluorescent label led only to a slight drop in inhibitory potency (see Table 1, entry 6).

The usefulness of building block **6** is further demonstrated in the on-resin synthesis of the biotinylated inhibitor **12** (Scheme 4), containing a photoactivatable group. This compound is designed to bind to the active site of MMPs and ADAMs, after which it can be covalently locked by irradiating the photo-cross-linker at 366 nm.¹⁴ The biotin moiety can then be used as a handle for affinity purification

(14) Two examples of similar compounds have recently been reported: (a) Saghatelian, A.; Jessani, N.; Joseph, A.; Humphrey, M.; Cravatt, B. F. *Proc. Natl. Acad. Sci.* **2004**, *101*, 10000–10005. (b) Chan, E. W. S.; Chattopadhyaya, S.; Panicker, R. C.; Huang, X.; Yao, S. Q. *J. Am. Chem. Soc.* **2004**, *126*, 14435–14446.

Scheme 4. Linear, On-Resin Synthesis of Biotinylated, Photo-Cross-Linker-Containing Inhibitor **12**^a



^a Mtt = 4-methyltrityl.

of the tagged enzymes, as well as for visualization by streptavidin after blotting.

Rink amide-bound Fmoc-Lys(Mtt) (**11**) was side-chain deprotected and then coupled to biotin. Next, the 6-amino-hexanoic (Ahx) spacer and the photo-cross-linker amino acid FmocPhe(tmd) were added. Finally, after removal of the Fmoc group and coupling of **6**, the entire construct was removed from the resin and concomitantly deblocked, to give **12** in an overall yield of 26% after HPLC purification.

Testing of compound **12** on MMP-12 and ADAM-17 revealed that it is a highly potent inhibitor of both enzymes (see Table 1, entry 7), as well as ADAM-10 (IC₅₀ = 114 nM). To validate it as a potential activity-based probe, **11** was incubated with recombinant ADAM-10, followed by irradiation at 366 nm, SDS-PAGE, and detection with streptavidin-alkaline phosphatase. The results of this experiment are shown in Figure 2.

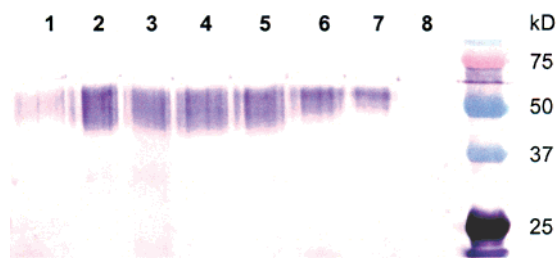


Figure 2. Blot of recombinant ADAM-10 (100 ng) incubated with photoactivatable inhibitor **12** at 2.5 μ M (except lane 2). The mixtures were irradiated with UV light (366 nm) for different times, followed by 10% SDS-PAGE and staining with streptavidin-AP. Lane 1: denatured ADAM-10 (preheated in SDS) and **12**. Lane 2: ADAM-10 + 500 nM of **12**. Lane 3: ADAM-10 + **12** + TAPI-2 (100 μ M). Lanes 4–8: ADAM-10 + **12** with decreasing irradiation times of 60, 30, 15, 5, and 0 min, respectively.

Clearly, compound **12** is able to irreversibly bind ADAM-

10 in an activity-based manner, as evidenced by the weak staining after preheating the enzyme (lane 1) in comparison with active ADAM-10 (lane 2). The binding efficiency of **12** is shown in lane 3, where a 40-fold excess of the generic MMP/ADAM inhibitor TAPI-2 only slightly decreases the observed staining. Figure 2 also shows that the covalent labeling is light dependent because no labeling is observed without irradiation (lane 8) and the maximum labeling occurs after approximately 30 min of irradiation. These facts make compound **12** a promising candidate for activity-based profiling of MMPs and ADAMs.

In conclusion, we have shown a straightforward solid-phase synthesis of succinylhydroxamate peptides using the readily accessible building block **6**. Potent, free-amine-containing MMP and ADAM inhibitors were conveniently synthesized and could be functionalized with a fluorescent label. Photoactivatable inhibitor **12** was efficiently synthesized entirely on the solid phase and shown to be suited for activity-based covalent labeling of a model ADAM. This synthetic strategy is a convenient alternative for the recently reported methodology⁸ and can be highly useful for the rapid, efficient synthesis of libraries of hydroxamate peptides. When

conjugated to a solid support via their free amines, compounds **8a–d** can be used in activity-based solid-phase extraction of MMPs or ADAMs.¹⁵ Compound **10** may be used for fluorescent staining of active MMPs and ADAMs. Compound **12** nicely complements recently disclosed activity-based probes for MMPs.¹⁴ The use of these compounds as tools in activity-based profiling is currently ongoing.

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Supporting Information Available: Detailed experimental procedures and full analytical data for all new compounds and details of the biochemical evaluations. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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