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Design of Peptide Hydroxamate-Based Photoreactive Activity-Based Probes of Zinc-Dependent Metalloproteases

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Metalloproteases (ADAMs, MMPs) are multidomain proteins that play key roles in extracellular matrix remodelling and degradation, in cell–cell and cell–matrix interactions and in the proteolytic liberation of membrane-anchored proforms of cytokines and growth factors, the so-called ectodomain shedding. In this work we describe the development of photoactivatable activity-based probes with which active metalloproteases can be visualised. Our probes are based on the succinyl hydroxamate motif and differ in the positioning of the trifluoromethylphenyldiazirine photoreactive group. We demonstrate that directing the photoactivatable group towards the S1′ pocket yields activity-based probes more effective than the corresponding probe with the photoactivatable group directed towards the S2′ pocket.

Introduction

Matrix metalloproteases (MMPs) and A Disintegrin And Metalloproteases (ADAMs) are catalytically active Zn^{2+}-dependent proteins that belong to the metzincin superfamily. Members of the MMP and ADAM families contain the consensus zinc-binding catalytic sequence HEXGHXXGXXHD in their metalloprotease domain.[1] MMPs have important physiological functions related to the degradation and turnover of extracellular matrix components, such as collagens and fibronectin.[2–5] ADAMs are membrane-anchored metalloproteases that display adhesive properties through their integrin-binding disintegrin domain and interaction of the cysteine-rich region with glycoproteins[6] and extracellular matrix proteins (for instance, fibronectin).[7] Their proteolytic activity is involved in so-called ectodomain shedding and is exemplified by the release of soluble TNFα from the cell membrane by ADAM-17 (TACE, TNF-alpha converting enzyme),[8] the release of heparin-binding epidermal growth factor by ADAM-9[9] and the α-secretase function of ADAM-10 in amyloid precursor protein (APP) processing[10] as well as the convertase activity of the same enzyme in the Notch/delta signaling pathway.[11] Deregulation of metalloprotease catalytic activity has been linked to inflammatory processes such as arthritis,[12] inflammatory bowel disorder,[7] Alzheimer's disease,[13] cancer[14,15] and cardiac hypertrophy.[16]

Conventional proteomics approaches to determine the relation of metalloproteases to disease states are limited by the fact that they take the total protein amount into account, whereas in many cases the functionality, that is the catalytic activity, is the relevant parameter. Several elegant methods to determine proteolytic activity in biological samples have been developed, such as zymography and activity-based ELISA.[17,18] Although these approaches visualise and quantify active proteases, application to a family-wide proteomics approach is difficult. Substrate specificity in zymography (for instance, gelatinases in gelatinzymography) and antibody specificity in ELISA make that both techniques are inherently limited to specific enzymes. Due to these limitations, there is a growing interest in the development of family-wide functional proteomics probes. Considerable progress have been made in the development and application of activity-based probes targeting cysteine proteases,[19–22] serine hydrolases[23–26] and proteasome subunits.[27–29] In these proteases a side-chain residue (serine, cysteine, threonine) acts as the nucleophilic species involved in amide-bond cleavage that is amenable to covalent and irreversible modification by installment of an appropriate electrophilic trap in the activity-based probes. MMPs and ADAMs employ a water molecule as the nucleophile in their active site, which precludes the use of such an electro-

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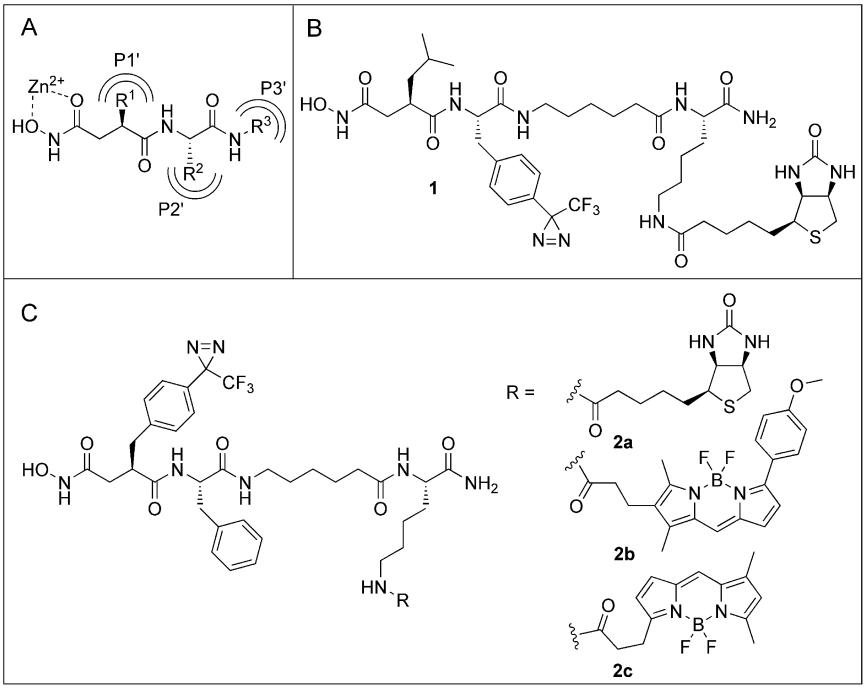
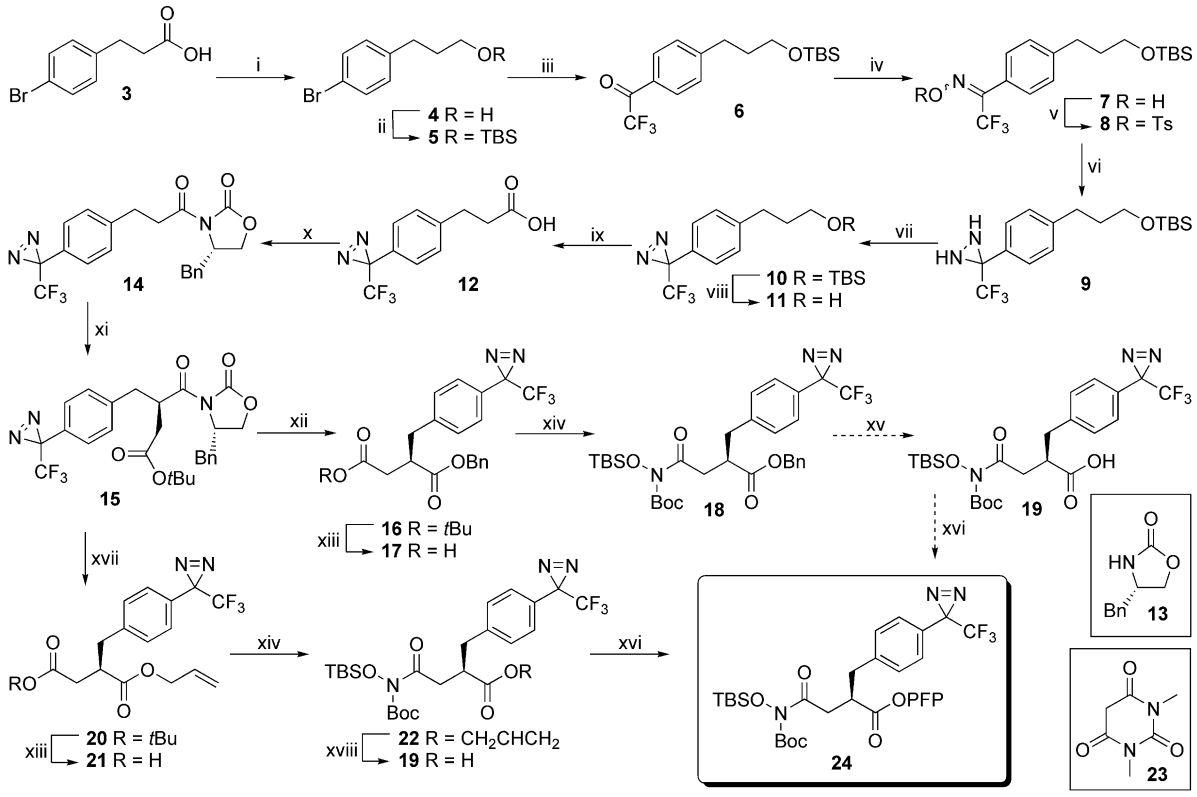


Figure 1. A) Generic binding mode of N-terminal peptide hydroxamates to metalloprotease active sites. B) Peptide hydroxamate-based activity-based metalloprotease probe from our previous studies with the photoactivatable group at P2′.[32] C) Compounds targeted in this study and featuring the photoactivatable group at the P1′ site.

philic trap. Photoaffinity labeling represents an alternative way to introduce tags into the active site of metalloproteases.[30,31] In this context, we recently reported[32] the synthesis of peptide hydroxamate **1** featuring both a biotin and a trifluoromethyldiazirine moiety (Figure 1, B). Upon incubation of purified recombinant ADAM-10 and subsequent irradiation with UV light (366 nm) the metalloprotease was covalently and irreversibly modified, as was evidenced by SDS PAGE of the denatured protein followed by streptavidin blotting. The efficiency of the photoaffinity labeling however proved rather modest. This raised the question whether the photoactivatable group would be better directed towards the S1′ pocket, rather than the S2′ pocket (see part A of Figure 1 for a general picture of the binding mode of N-terminal peptide hydroxamate-based metalloprotease inhibitors). Examination of the available 3-dimensional structures of metalloprotease-inhibitor complexes indicates that the S1′ pocket in general should be able to accommodate rather bulky hydrophobic groups at this position.[33–36] We decided to address this issue by the synthesis of peptide hydroxamate **2a** (Figure 1, C) with the photoactivatable group at P1′, and compare its MMP/ADAM labeling efficiency to that of probe **1** having the photoactivatable group at P2′. We here report an efficient synthesis of the required diazirine-modified succinyl hydroxamate building block and its application in the synthesis of activity-based probe **2a** along with a pair of fluorescent analogues **2b** and **2c**. We demonstrate in a head-to-head comparison that **2a** indeed is the more efficient photoactivatable MMP/ADAM activity-based probe towards a range of recombinant, purified metalloproteases.

Results and Discussion

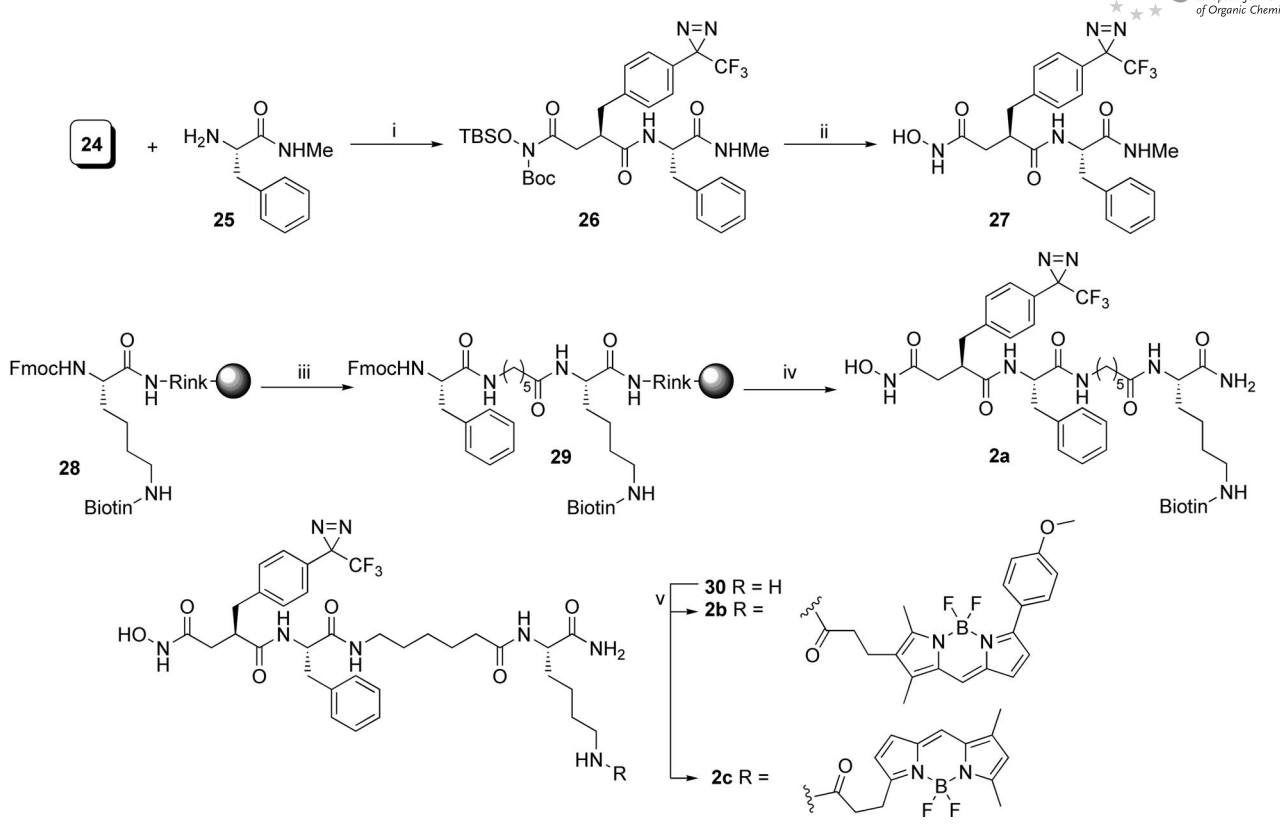
In our previous work,[32] on the synthesis of ABP **1**, we employed a chiral, L-leucine mimetic succinyl hydroxamate in which the hydroxamic acid moiety was protected with acid-labile protective groups [*O*(TBS)-*N*(Boc)-R] and the carboxylate activated as the pentafluorophenyl ester. This building block can be readily incorporated in Fmoc-based solid-phase peptide synthesis protocols as the final building block after which the immobilized peptide hydroxamate is in one step cleaved from the resin and concomitantly deprotected. Our strategy appeared of general use as we demonstrated by the construction of a peptide hydroxamate library[37] and we therefore decided to apply a similar strategy for the construction of our target compounds. This required the synthesis of trifluoromethylphenyldiazirine functionalised, pentafluorophenyl-activated ester **24** (Scheme 1), which we prepared as follows. Reduction of 4-bromophenylpropionic acid (**3**) ($LiAlH_4$) provided alcohol **4** that was transformed into TBS ether **5**. At this stage, the trifluoroacetyl moiety was introduced by first lithiation and subsequent addition of 1-trifluoroacetylpiperidine. Refluxing the resulting ketone **6** and hydroxylamine in pyridine afforded oxime **7** as an *E*/*Z* mixture. The hydroxy group in **7** was converted into the tosylate **8** after which reaction with liquid ammonia under 8 bar at room temperature led to the formation of diaziridine **9**. Oxidation of the diaziridine using iodine provided diazirine **10**. Acidolysis of the TBS ether followed by biphasic TEMPO/BAIB oxidation of the resulting alcohol **11**[38] provided carboxylic acid **12** in 57% overall yield over the nine steps.



Scheme 1. Reagents and conditions: (i) $LiAlH_4$, Et_2O, 0 °C, quant.; (ii) TBSCl, imidazole, DMF, quant.; (iii) *n*BuLi, 1-trifluoroacetylpiperidine, Et_2O, –78 °C→r.t., 76%; (iv) $HONH_2$·HCl, pyridine, Δ, 93%; (v) TsCl, Et_3N, DMAP, DCM, 97%; (vi) NH_3, Et_2O, 8 bar, 95%; (vii) I_2, Et_3N, MeOH, 94%; (viii) HCl, H_2O, MeOH, 97%; (ix) TEMPO, BAIB, DCM, H_2O, 96%; (x) (a) $(ClCO)_2$, DMF, DCM; (b) **13**, *n*BuLi, THF, 0 °C, 82%; (xi) LiHMDS, *tert*-butyl bromoacetate, THF, –78 °C, 70%; (xii) BnOH, *n*BuLi, THF, 0 °C, 82%; (xiii) TFA, DCM, 97%; (xiv) (a) $(ClCO)_2$, DMF, DCM; (b) TBSONHBoc, *n*BuLi, THF, 27% for **18** and 77% for **22**; (xv) several conditions, no yield; (xvi) PFPOH, EDCI, DCM, 88% over 2 steps; (xvii) allyl alcohol, *n*BuLi, THF, 0 °C, 65%; (xviii) **23**, $Pd(PPh)_4$, THF.

The route of synthesis continued by condensation of **12**, via its acyl chloride, with the lithium salt of chiral auxiliary **13**.[39] Deprotonation of the resulting intermediate **14** followed by enantioselective alkylation with *tert*-butyl bromoacetate afforded succinate **15** as the single observed diastereomer in 57% yield over the two steps. In line with our previous studies[32] we substituted the Evans template with lithium benzyl alcoholate to give diester **16**. Selective acidic removal of the *tert*-butyl group gave carboxylic acid **17** which was transformed into fully protected succinyl hydroxamate **18** by first transformation into the corresponding acyl chloride and next reaction with the lithiate of *N*-Boc-*O*-TBS-hydroxylamine.[40] We previously found[32] that condensation of *N*-Boc-*O*-TBS-hydroxylamine with related acyl chlorides proceeded well under the agency of two equivalents of 4-dimethylaminopyridine (DMAP) as the base. However, this procedure proved less efficient in the transformation aimed for here, and optimal results were obtained by adding dropwise the lithiate of *N*-Boc-*O*-TBS-hydroxylamine to a THF solution of the acyl chloride. Unfortunately deprotection of the benzyl ester to carboxylic acid **19** failed under the conditions attempted [Pd/C, H_2 or $Pd(OAc)_2$, Et_3SiH, Et_3N], either because the hydrogenation of the benzyl proceeded sluggishly or the diazirine was reduced concomitantly to the diaziridine. Rather than searching for a protocol in which the diaziridine is oxidised back to the diazirine after hydrogenation, we opted to adapt our protective group scheme, as follows. Reaction of compound **15** with the lithium salt of allyl alcohol afforded allyl ester **20**.[41] Partial deprotection and condensation with the protected hydroxylamine as described before gave succinyl hydroxamate **22**. Now, the allyl ester was removed by reaction with tetrakis(triphenylphosphane)palladium in the presence of *N*,*N*′-dimethylbarbaturic acid (**23**) as the allyl scavenger, providing carboxylate **19**. The latter was converted into the key intermediate, pentafluorophenyl ester **24**, in 88% yield over the last two steps. The choice for chiral alkylation by means of Evans template chemistry was based on our previous work in which we showed that alkylation and substitution of the Evans template, both of which entail strong basic conditions, gave optically pure products.[32] Indeed, in the here presented synthesis we did not observe any kind of epimerization. The chiral outcome of these steps however can vary with the type of side chain used and the use of a different kind of chiral auxiliary, for example the thiazolidinethione derivatives, which are known to display a better leaving group ability,[42] may then prove necessary.

The construction of peptide hydroxamates incorporating the chiral succinic acid derivative is depicted in Scheme 2. In the first instance we reacted pentafluorophenyl ester **24**



Scheme 2. Reagents and conditions: (i) DMF, 45%; (ii) TFA/H_2O, 95:5 (v:v), 67%; (iii) (a) 20% piperidine/DMF; (b) Fmoc-Ahx-OH, HCTU, DIEA, NMP; (c) 20% piperidine/DMF; (d) Fmoc-Phe-OH, HCTU, DIEA, NMP; (iv) (a) 20% piperidine/DMF; (b) **24**, DIEA, NMP; (c) TFA/H_2O/TIS, 95:2.5:2.5 (v:v:v), 30% from **28**; (v) Bodipy(Tmr)-OSu or Bodipy(FL)-OSu, DIEA, DMF, 31% and 55%, respectively.

with L-phenylalanine methylamide (**25**)[43] in DMF to give bisamide **26**, which was finally deprotected [TFA/H_2O, 95:5 (v:v)] to the free hydroxamic acid **27** in 30% yield over the two steps.[44] This experiment at once established that building block **24** is compatible with the peptide coupling/global deprotection conditions envisioned and delivered a tag-free analogue of our target compounds for control experiments (see below). The target compound **2a** was prepared by Fmoc-based solid-phase peptide synthesis starting with RINK amide-bound Fmoc-biocytin **28**. Standard Fmoc-based solid-phase peptide synthesis afforded immobilized tripeptide **29**, which was transformed in three steps (first Fmoc removal, then condensation with pentafluorophenyl ester **24** and finally removal from the solid support with concomitant global deprotection) into peptide hydroxamate **2a** in 30% overall yield after RP-HPLC purification and based on **28**. In a similar fashion, but employing FmocLys-(Boc) instead of Fmoc-biocytin, peptide hydroxamate **30** was prepared. Treatment of **30** with either Bodipy(TMR)-OSu[45] or Bodipy(FL)-OSu[46] gave compounds **2b** and **2c**, respectively, in 31% and 55% yield.

We next set out to compare the labeling efficiency of probes **1** and **2a** against a panel of MMPs and ADAMs. In a first experiment we assessed the inhibitory potency of the two probes against MMP-9, MMP-12, ADAM-10 and ADAM-17 (Table 1). Although the four enzymes are inhibited in the nanomolar range by both compounds, there are some differences in potency. The values differ especially for ADAM-17, for which hydroxamate **1** appeared about 25 fold more potent compared to **2a**. Interestingly, both compounds appear equally efficient in labeling ADAM-17, as is evidenced from Figure 2. In this experiment, recombinant and purified ADAM-17 was exposed to either **1** or **2a** and UV light prior to denaturation, SDS PAGE and streptavidin blotting. It seems that inhibitory efficiency is not directly correlated to photoaffinity labeling and we argue that the mechanism by which the trifluoromethyldiazirine dissociates and reacts upon irradiation is behind this observation. Upon photoexcitation nitrogen is expulsed with concomitant formation of a highly reactive carbene that will insert in the first available X–H bond.[47] In case an (active site) amino acid is nearby effective photoaffinity labeling is the expected result, whereas poor labeling will occur in case the photoreactive group is solvent (water) exposed. We tentatively conclude that the diazirine moiety in **2a** is bound more tightly to ADAM-17 than the one in **1**, even though the latter compound is the more potent inhibitor. Perusal of a panel of ten recombinant and purified MMPs and three more ADAMs reveals that, in general, peptide hydroxamate **2a** is the more effective affinity label (Figure 2). In each case compound **2a** is at least as effective (compare for instance the data obtained for ADAM-9 and ADAM-10) and often in fact provides a signal where compound **1** does not (see for instance the results obtained for MMP-13 and

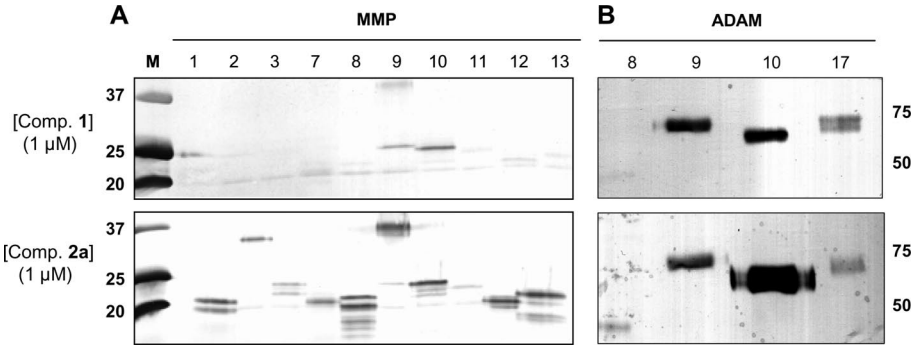


Figure 2. Photoaffinity labeling of MMPs (A) and ADAMs (B) using probe **1** (upper panels) and probe **2a** (lower panels) at 1 μM. The modified proteins were visualized by anti-biotin Western blots with streptavidin-alkaline phosphatase. Ten recombinant MMP and four recombinant ADAM proteases (4 pmol each; numbers above the lanes correspond to the respective protease) were studied. Multiple biotinylated bands in the recombinant MMPs indicate auto-degradation. M: molecular weight marker.

ADAM-8). Interestingly, multiple bands appear for some of the MMP labeling experiments (see for instance, MMP-1 and MMP-8 treated with **2a**), with the band corresponding to the highest molecular weight in each case corresponding to the molecular weight of the full-length MMP at hand. These bands in all likelihood are the result of auto-degradation, in which unmodified MMP processes its photoaffinity labeled counterpart. From these experiments we conclude that positioning the photoactivatable group at P1′ as in **2a** indeed gives a comparatively more potent MMP/ADAM photoactivatable activity-based probe.

Table 1. IC_{50} values (in nM) of compounds **1** and **2a**.

	MMP-9	MMP-12	ADAM-10	ADAM-17
1	25.1	3.60[a]	114[a]	20.6[a]
2a	24.2	12.5	54.1	490

[a] From reference.[32]

In order to prove that labeling is activity-dependent, aliquots of MMP-9 and MMP-12 were incubated with either the natural inhibitor, TIMP-1[48] or the non-biotinylated inhibitor **27** (Figure 3). In the presence of equimolar amounts (relative to **2a**) of TIMP-1 neither MMP-9 nor MMP-12 were detectably labeled. Preincubation of MMP-9 or MMP-12 with twofold molar excess, relative to **2a**, of the non-biotinylated inhibitor **27** also effectively abolished labeling. Taken together, these data provide strong evidence that photoaffinity labeling is activity-dependent and that labeling occurs most likely in the active site of the enzymes.

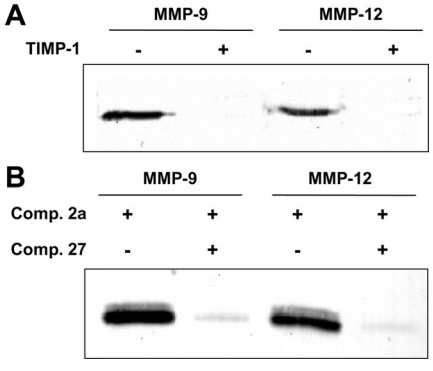


Figure 3. Activity-dependence of photoaffinity labeling of MMP-9 and MMP-12 (4 pmol each) with probe **2a** (200 nM final concentration) as shown by competition with (A) an equimolar amount of the endogenous MMP inhibitor TIMP-1 (200 nM final concentration) and with (B) a twofold molar excess of compound **27** (400 nM final concentration).

Conclusions

In summary, we have described the development of an efficient photoactivatable activity-based probe with which a broad panel of MMPs and ADAMs can be covalently and irreversibly modified in an activity-dependent fashion. Our work demonstrates that the enantioselective synthesis strategy we previously reported[32,37] for the preparation of an enantiomerically pure, alkylated succinyl hydroxamate is also effective, in adapted form, for the synthesis of the functionally more challenging key building block **24**. Further, our hypothesis that placing the photoactivatable trifluoromethyldiazirine in the P1′ position would lead to more effective activity-based probes proved valid. We are now faced with the challenge to detect MMPs and ADAMs in their natural environment and at natural abundance levels in a photoactivatable activity-based proteomics profiling experimental setting.[30] The ability to prepare, with relative ease, peptide hydroxamates analoguous to compounds **1** and **2a** such as Bodipy derivatives **2b**,**c** may well be indispensable in reaching this research objective, on which we will report in the future.

Experimental Section

Syntheses

General Methods: Tetrahydrofuran was distilled from $LiAlH_4$ prior to use. Acetonitrile (ACN), dichloromethane (DCM), dimethylformamide (DMF), *N*-methyl-2-pyrrolidone (NMP), methanol (MeOH), piperidine, diisopropylethylamine (DIEA) and trifluoroacetic acid (TFA) were of peptide synthesis grade, purchased at Biosolve, and used as received. All general chemicals (Fluka, Acros, Merck, Aldrich, Sigma) were used as received. Rink amide MBHA resin (0.64 mmol/g) was purchased at Novabiochem, as well as all

appropriately protected amino acids [Fmoc-Phe-OH, Fmoc-Ahx-OH, Fmoc-Lys(Mtt)-OH]. *O*-(6-Chloro-1*H*-benzotriazolyl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HCTU) was purchased at Iris Biotech (Marktrewitz, Germany). Traces of water were removed from reagents used in reactions that require anhydrous conditions by co-evaporation with toluene. Solvents that were used in reactions were stored over 4 Å molecular sieves, except for methanol and acetonitrile, which were stored over 3 Å molecular sieves. Molecular sieves were flame-dried before use. Unless noted otherwise all reactions were performed under argon. Column chromatography was performed on silica gel (purchased from Screening Devices b.v.; particle size 40–63 μm, pore diameter of 60 Å). The eluents toluene, ethyl acetate and petroleum ether (40–60 °C boiling range) were distilled prior to use. TLC analysis was conducted on Merck aluminium sheets (Silica gel 60 F_{254}). Compounds were visualized by UV absorption (254 nm), by spraying with a solution of $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ (25 g/L) and $(NH_4)_4Ce(SO_4)_4{\cdot}2H_2O$ (10 g/L) in 10% sulfuric acid, a solution of $KMnO_4$ (20 g/L) and K_2CO_3 (10 g/L) in water, or ninhydrin (0.75 g/L) and acetic acid (12.5 mL/L) in ethanol, where appropriate, followed by charring at ca. 150 °C. ^{1}H- and ^{13}C-NMR spectra were recorded with a Bruker AV-400 (400 MHz), a Bruker AV-500 (500 MHz) or a Bruker DMX-600 (600 MHz) spectrometer. ^{19}F NMR spectra were recorded with a Bruker AV-200 (200 MHz) or a Bruker DMX-400 (400 MHz). Chemical shifts are given in ppm (δ) relative to tetramethylsilane, CD_3OD, [D_6]DMSO, $CDCl_3$ or $CFCl_3$ as internal standard. High-resolution mass spectra were recorded by direct injection [2 μL of a 2 μM solution in water/acetonitrile, 1:1 (v/v) and 0.1% formic acid] on a mass spectrometer (Thermo Finnigan LTQ Orbitrap) equipped with an electrospray ion source in positive mode (source voltage 3.5 kV, sheath gas flow 10, capillary temperature 250 °C) with resolution R = 60000 at m/z 400 (mass range m/z = 150–2000) and dioctyl phthalate (m/z = 391.28428) as a "lock mass". The high-resolution mass spectrometer was calibrated prior to measurements with a calibration mixture (Thermo Finnigan). Optical rotations $[\alpha]_D^{23}$ were recorded with a Propol automatic polarimeter.

General Procedure for Solid-Phase Peptide Synthesis: Fmoc Rink Amide MBHA resin (0.64 mmol/g) was used as received. Prior to first use, it was washed twice with DMF, twice with methanol and twice with DCM. Fmoc deprotection was performed by shaking the resin in a 20% piperidine/DMF (v/v) stock solution for 20 min. The resin was washed twice with DMF and twice with DCM after every coupling and deprotection step. The first amino acid was loaded by reacting the resin with 4 equiv. of HCTU, 4 equiv. of amino acid and 8 equiv. DIEA (0.45 M stock solution in NMP). The amino acid was pre-activated in solution (5 min) before adding it to the resin and shaking the resin for 1 h (standard coupling protocol). Loading was determined by UV spectroscopy at 300 nm of a freshly prepared Fmoc-deprotected resin sample. A capping step was performed by shaking the resin for 10 min with 0.45 M acetic anhydride and 0.45 M DIEA/NMP solution. Mtt deprotection was done by shaking repeatedly in a 1% TFA/DCM solution until the characteristic yellow colour of the Mtt cation did no longer appear (7–12 times, 2 min each). After Mtt cleavage, immediately before the following coupling step, the resin was washed with a 0.45 M DIEA stock solution in NMP. The coupling and deprotection reactions were checked on the presence of free amines by performing a Kaiser test. Before cleaving the peptide from the resin, it was washed 5 times alternatingly with DCM and MeOH. Cleavage from the solid support was done by shaking the resin in a TFA/H_2O/TIS, 95:2.5:2.5 (v/v/v) solution for 2 h, followed by filtration and rinsing the resin with a small portion of TFA/H_2O/TIS, 95:2.5:2.5 (v/v/v). The resulting filtrate was concentrated under reduced pressure and the product was purified by RP-HPLC.

LC-MS Analysis and HPLC Purifications. System A: LC-MS analysis was performed with a Finnigan Surveyor HPLC system with a Gemini C18 50 × 4.60 mm column (detection at 200–600 nm), coupled to a Finnigan LCQ Advantage Max mass spectrometer with ESI. The applied buffers were H_2O, ACN and 1.0% aq. TFA.

System B: HPLC purifications were performed with a Gilson HPLC system coupled to a Phenomenex Gemini 5 μm 250 × 10 mm column and a GX281 fraction collector. The applied buffers were: 0.1% aq. TFA and ACN. Appropriate fractions were pooled, and concentrated in a Christ rotary vacuum concentrator overnight at room temperature at 0.1 mbar.

Compounds

3-(4-Bromophenyl)propanol (4): Commercially available 3-(4-bromophenyl)propanoic acid (**3**, 10.8 g, 46.22 mmol) was dissolved in Et_2O (250 mL) and cooled to 0 °C. To this solution was carefully added $LiAlH_4$ (1.3 equiv., 60 mmol, 2.28 g) in portions. The reaction was slowly warmed to room temperature in 1 h after which TLC analysis indicated a completed reaction. 1 M aq. HCl (200 mL) was slowly added and the layers were separated. The organic layer was extracted with 1 M aq. HCl, saturated aq. $NaHCO_3$ (twice) and brine (200 mL, each), dried with $MgSO_4$ and concentrated under reduced pressure. The product was obtained as a colourless oil (yield: 9.9 g, 46.2 mmol, quant.). The spectroscopic data correspond with those reported in literature.[49]

[3-(4-Bromophenyl)propoxy](*tert*-butyl)dimethylsilane (5): To a solution of alcohol **4** (9.9 g, 46.2 mmol) in DMF (80 mL) were added imidazole (4.77 g, 69.3 mmol) and *tert*-butylchlorodimethylsilane (TBS-Cl) (7.82 g, 50.8 mmol). The reaction was stirred for 2 h after which TLC analysis indicated complete conversion. Deionised H_2O (300 mL) was added and the mixture was extracted 3 times with petroleum ether (200 mL). The combined organic layers were extracted with deionised H_2O (4 times) and brine (200 mL, each), dried with $MgSO_4$ and concentrated under reduced pressure. The product was obtained as a colourless oil (yield: 15.2 g, 46.2 mmol, quant.) and subjected to the next step without further purification. The spectroscopic data correspond with those reported in literature.[50]

1-{4-[3-(*tert*-Butyldimethylsilyloxy)propyl]phenyl}-2,2,2-trifluoroethanone (6): Bromide **5** (7.03 g, 21.36 mmol) was dissolved in Et_2O (100 mL) and cooled to –78 °C. *n*BuLi (26.7 mmol, 16.7 mL, 1.6 M in THF) was added dropwise and the solution was slowly warmed to room temperature at which it was stirred for 1 h. Then the mixture was cooled again to –78 °C and a solution of 1-trifluoroacetylpiperidine (4.2 g, 23.2 mmol) in Et_2O (5 mL) was added dropwise. The solution was slowly warmed to 0 °C in 2 h after which TLC analysis indicated a complete conversion. The reaction was quenched with saturated aq. NH_4Cl (100 mL) and the layers were separated. The organic layer was extracted with saturated aq. NH_4Cl, deionised water and brine, dried with $MgSO_4$ and concentrated under reduced pressure. The crude material was purified by column chromatography (10% → 30% toluene/petroleum ether) and the product was obtained as a colourless oil (yield: 5.63 g, 16.3 mmol, 76%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.00 (d, J = 8.0 Hz, 2 H, Ph), 7.37 (d, J = 8.0 Hz, 2 H, Ph), 3.64 (t, J = 6.4 Hz, 2 H, OCH_2), 2.79 (t, J = 7.6 Hz, 2 H, $PhCH_2$), 1.89–1.84 (m, 2 H, CH_2), 0.91 (s, 9 H, 3 × CH_3), 0.06 (s, 6 H, 2 × $SiCH_3$) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 151.3, 130.2, 129.3, 127.7, 116.8 (q, J = 290 Hz, CF_3), 61.4, 33.9, 32.4, 25.9, 18.2, –5.4 ppm. HRMS: calcd. for $C_{17}H_{25}F_3O_2Si$ $[M + H]^+$ 347.16487; found 347.16505.

1-{4-[3-(*tert*-Butyldimethylsilyloxy)propyl]phenyl}-2,2,2-trifluoroethanone Oxime (7): Ketone **6** (9.43 g, 27.2 mmol) was dissolved in pyridine (30 mL) and hydroxylamine hydrochloride (5.68 mmol, 81.7 mmol) was added. The mixture was stirred at reflux for 2 h after which TLC analysis indicated a complete conversion. The mixture was concentrated under reduced pressure and dissolved in EtOAc (100 mL) and 0.2 M aq. citric acid (100 mL). The layers were separated and the organic layer was extracted with 100 mL of 0.2 M aq. citric acid, deionised water and brine, dried with $MgSO_4$ and concentrated under reduced pressure. The crude product was obtained as a colourless oil (yield: 9.13 g, 25.3 mmol, 93%) as a *Z*/*E* mixture. A small amount was purified by column chromatography (5% → 10% EtOAc/petroleum ether) for characterization. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.51 (br. s, 1 H, OH), 8.25 (br. s, 1 H, OH), 7.44–7.39 (m, 2 H, Ph), 7.31–7.23 (m, 2 H, Ph), 3.67–3.62 (m, 2 H, OCH_2), 2.74–2.69 (m, 2 H, $PhCH_2$), 1.89–1.82 (m, 2 H, CH_2), 0.91 (s, 9 H, 3× CH_3), 0.06 (s, 6 H, 2× $SiCH_3$) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 147.4–146.5 (m, C=N), 144.9, 144.5, 129.2, 128.9, 128.9, 128.7, 128.6, 128.6, 128.4, 128.3, 128.2, 127.9, 123.7, 120.9 (q, *J* = 273 Hz, CF_3), 118.6 (q, *J* = 281 Hz, CF_3), 63.0, 62.8, 34.0, 33.9, 32.0, 31.9, 25.9, 18.4, –5.3, –5.5 ppm. HRMS: calcd. for $C_{17}H_{26}F_3NO_2Si$ $[M + H]^+$ 362.17577; found 362.17583.

1-{4-[3-(*tert*-Butyldimethylsilyloxy)propyl]phenyl}-2,2,2-trifluoroethanone *O*-Tosyl Oxime (8): Oxime **7** (9.13 g, 25.3 mmol) was dissolved in DCM (20 mL). To this solution were added Et_3N (5.26 mL, 37.95 mmol) and DMAP (60 mg, 0.5 mmol) after which TsCl (4.84 g, 25.3 mmol) in DCM (20 mL) was added dropwise over 1 h. After stirring the mixture at room temperature for 30 min TLC analysis indicated a complete conversion. 0.2 M aq. citric acid (100 mL) was added and the layers were separated. The organic layer was extracted with 0.2 M aq. citric acid (100 mL) and brine, dried with $MgSO_4$ and concentrated under reduced pressure yielding the crude product (*Z*/*E* mixture) as a colourless oil (yield: 12.59 g, 24.4 mmol, 97%). A small amount was purified by column chromatography (1.5% → 7.5% EtOAc/petroleum ether) for characterization. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.91–7.88 (m, 2 H, Ph), 7.39–7.25 (m, 6 H, Ph), 3.66–3.62 (m, 2 H, OCH_2), 2.73 (t, *J* = 7.8 Hz, 2 H, $PhCH_2$), 2.48 (s, 3 H, $PhCH_3$), 2.46 (s, 3 H, $PhCH_3$), 1.88–1.83 (m, 2 H, CH_2), 0.91 (s, 9 H, 3× CH_3), 0.90 (s, 9 H, 3× CH_3), 0.06 (s, 6 H, 2× $SiCH_3$), 0.05 (s, 6 H, 2× $SiCH_3$) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 153.8 (q, *J* = 34.4 Hz, C=N), 146.8, 146.6, 146.0, 145.9, 131.5, 131.2, 129.7, 129.1, 128.9, 129.8, 128.7, 128.4, 121.8, 119.7 (q, *J* = 276 Hz, CF_3), 61.9, 61.8, 33.8, 33.8, 32.0, 31.9, 25.8, 21.5, 18.1, –5.5 ppm. HRMS:: calcd. for $C_{24}H_{32}F_3NO_4SSiH^+$ 516.18462; found 516.18443, calcd. for $C_{24}H_{32}F_3NO_4SSi$ $[M + Na]^+$ 538.16656; found 538.16655.

3-{4-[3-(*tert*-Butyldimethylsilyloxy)propyl]phenyl}-3-(trifluoromethyl)diaziridine (9): Tosylate **7** (12.6 g, 24.4 mmol) was dissolved in Et_2O (30 mL) in an autoclave and cooled to –78 °C. Freshly condensed ammonia (≈ 5 mL) was added and the autoclave was closed and warmed to room temperature. The pressure inside increased to 8 bar. After 5 h the autoclave was cooled to –78 °C and opened. Deionised water (40 mL) was carefully added and the mixture was warmed to room temperature. The layers were separated and the organic layer was extracted with deionised water (3× 50 mL) and brine, dried with $MgSO_4$ and concentrated under reduced pressure. The crude diaziridine was obtained as a colourless oil (yield: 8.32 g, 23.1 mmol, 95%). A small amount was purified by column chromatography (2% → 11% EtOAc/petroleum ether) for characterization. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.51 (d, *J* = 8.0 Hz, 2 H, Ph), 7.24 (d, *J* = 8.0 Hz, 2 H, Ph), 3.63 (t, *J* = 6.2 Hz, 2 H, OCH_2), 2.77 (d, *J* = 8.8 Hz, 1 H, NH), 2.71 (t, *J* = 7.8 Hz, 2 H, $PhCH_2$), 2.20 (d, *J* = 8.8 Hz, 1 H, NH), 1.86–1.81 (m, 2 H, CH_2), 0.90 (s, 9 H, 3× CH_3), 0.05 (s, 6 H, 2× $SiCH_3$) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 144.3, 129.1, 128.8, 127.9, 123.6 (q, *J* = 277 Hz, CF_3), 61.9, 57.7 (q, *J* = 35.7 Hz, CNHNH), 34.0, 31.7, 25.7, 18.1, –5.6 ppm. HRMS: calcd. for $C_{17}H_{27}F_3N_2OSi$ $[M + H]^+$ 361.19175; found 361.19183.

3-{4-[3-(*tert*-Butyldimethylsilyloxy)propyl]phenyl}-3-(trifluoromethyl)-3*H*-diazirine (10): Et_3N (46.2 mmol, 6.4 mL) was added to a solution of diaziridine **8** (8.32 g, 23.1 mmol) in MeOH (25 mL). Then I_2 (23.1 mmol, 5.86 g) was added in portions, letting the mixture decolorize after every portion. Eventually the reaction mixture did not decolorize anymore and the mixture was stirred for another 30 min. TLC analysis indicated a completed reaction and a 10% w/w aq. citric acid solution (100 mL) was added. The mixture was extracted twice with Et_2O (150 mL) and the combined organic layers were extracted with 10% w/w aq. citric acid (100 mL), a saturated aq. $NaHSO_3$ solution (100 mL), deionised water (100 mL) and brine (100 mL), dried with $MgSO_4$ and concentrated under reduced pressure. The crude diazirine was obtained as a colourless oil (yield: 7.77 g, 21.7 mmol, 94%). A small amount was purified by column chromatography (1% → 2.5% EtOAc/petroleum ether) for characterization. ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.22 (d, *J* = 8.4 Hz, 2 H, Ph), 7.10 (d, *J* = 7.8 Hz, 2 H, Ph), 3.61 (t, *J* = 6.3 Hz, 2 H, OCH_2), 2.69 (t, *J* = 7.5 Hz, 2 H, $PhCH_2$), 1.82–1.79 (m, 2 H, CH_2), 0.90 (s, 9 H, 3× CH_3), 0.05 (s, 6 H, 2× $SiCH_3$) ppm. ^{13}C NMR (150 MHz, $CDCl_3$): δ = 144.2, 129.0, 126.6, 126.5, 122.4 (q, *J* = 273 Hz, CF_3), 61.9, 34.2, 31.8, 28.4 (q, *J* = 40 Hz, CNN), 25.9, 18.3, –5.5 ppm. HRMS: calcd. for $C_{17}H_{25}F_3N_2OSi$ $[M + H]^+$ 359.17610; found 359.17616.

3-{4-[3-(Trifluoromethyl)-3*H*-diazirin-3-yl]phenyl}propan-1-ol (11): Compound **10** (7.78 g, 21.7 mmol) was dissolved in MeOH (50 mL) and concentrated HCl [37% (w/v), 3 mL] was added. The reaction mixture was stirred at room temperature until TLC analysis revealed a completed reaction after 2 h. The solvent was evaporated under reduced pressure and the residue was dissolved in Et_2O (200 mL). The resulting solution was extracted twice with a saturated aq. $NaHCO_3$ solution (150 mL) and brine (100 mL), dried with $MgSO_4$ and concentrated under reduced pressure. The crude mixture was purified by column chromatography (10% → 60% EtOAc/petroleum ether) and the pure alcohol **11** was obtained as a colourless oil (yield: 5.13 g, 21.0 mmol, 97%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.25 (d, *J* = 8.0 Hz, 2 H, Ph), 7.12 (d, *J* = 8.0 Hz, 2 H, Ph), 3.66 (t, *J* = 6.4 Hz, 2 H, OCH_2), 2.72 (d, *J* = 7.8 Hz, 2 H, $PhCH_2$), 1.90–1.83 (m, 2 H, CH_2), 1.43 (br. s, 1 H, OH) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 143.7, 129.1, 126.5, 126.4, 122.2 (q, *J* = 263 Hz, CF_3), 61.4, 33.7, 31.6, 28.2 (q, *J* = 40.2 Hz, CNN) ppm. HRMS: calcd. for $C_{11}H_{11}F_3N_2O$ $[M + H]^+$ 245.08962; found 245.08978.

3-{4-[3-(Trifluoromethyl)-3*H*-diazirin-3-yl]phenyl}propanoic Acid (12): Alcohol **11** (2.1 g, 8.6 mmol) was dissolved in a mixture of DCM (25 mL) and deionised H_2O (12.5 mL). To this mixture were added 2,2,6,6-tetramethylpiperidinooxy (TEMPO) (0.1 equiv., 0.86 mmol, 134 mg) and [bis(acetoxy)iodo]benzene (BAIB) (2.5 equiv., 21.5 mmol, 6.92 g). The reaction was stirred overnight at room temperature after which TLC analysis revealed a completed reaction. A saturated aq. $Na_2S_2O_3$ solution (100 mL) was added and the mixture was vigorously stirred for 5 min. EtOAc (150 mL) was added and the layers were separated. The organic layer was extracted with 1 M aq. HCl and brine (100 mL), dried with $MgSO_4$ and concentrated under reduced pressure. The crude material was purified by column chromatography (10% → 30% EtOAc/petroleum ether) and the product was obtained as a colourless solid (yield: 2.13 g, 8.23 mmol, 96%). ^{1}H NMR (600 MHz,

$CDCl_3$): δ = 11.81 (br. s, 1 H, CO_2H),7.22 (d, J = 9.0 Hz, 2 H, Ph), 7.11 (d, J = 9.6 Hz, 2 H, Ph), 2.94 (t, J = 8.1 Hz, 2 H, CH_2), 2.65 (t, J = 8.1 Hz, 2 H, CH_2) ppm. ^{13}C NMR (150 MHz, $CDCl_3$): δ = 179.4, 142.0, 128.8, 127.2, 126.6, 122.2 (q, J = 273 Hz, CF_3), 35.2, 30.1, 28.4 (q, J = 40.5 Hz, CNN) ppm. HRMS: calcd. for $C_{11}H_9F_3N_2O_2$ $[M + H]^+$ 259.06889; found 259.06909.

(*S*)-4-Benzyl-3-(3-{4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]phenyl}-propanoyl)oxazolidin-2-one (14): Carboxylic acid **12** (1.04 g, 4.03 mmol) was dissolved in DCM (25 mL) and DMF (1 drop) was added. The mixture was cooled to 0 °C and oxalyl chloride (1.7 mL, 20 mmol) was added dropwise. The reaction was warmed to room temperature and stirred for 30 min after which gas formation ceased. Toluene was added and the mixture was concentrated in vacuo followed by coevaporation with toluene (twice). The crude product was subjected to the next step without further purification. *n*BuLi (2.75 mL of a 1.6 M solution in THF, 4.4 mmol) was put into a flask under an argon atmosphere and cooled to 0 °C. To this was added dropwise a solution of (*S*)-4-benzyloxazolidin-2-one[39] (**13**, 0.78 g, 4.43 mmol) in THF (15 mL) and the mixture was stirred at 0 °C for 15 min, thereby forming a white precipitant. Then the freshly prepared acyl chloride in THF (10 mL) was added to the reaction mixture dropwise and the mixture was slowly warmed to room temperature. After 2 h at room temperature, TLC analysis showed complete consumption of the starting material. To the mixture was added deionised water (50 mL) and it was extracted twice with EtOAc (50 mL). The combined organic layers were extracted with a saturated aq. solution of $NaHCO_3$ (2 × 50 mL) and brine (50 mL), dried with $MgSO_4$ and concentrated under reduced pressure. The obtained crude material was purified by column chromatography (10% → 30% EtOAc/petroleum ether) and the product was obtained as a colourless solid (yield: 1.38 g, 3.31 mmol, 82%). ^1H NMR (600 MHz, $CDCl_3$): δ = 7.32–7.25 (m, 5 H, Ph), 7.16 (d, J = 7.2 Hz, 2 H, Ph), 7.13 (d, J = 8.4 Hz, 2 H, Ph), 4.67–4.63 (m, 1 H, NCH), 4.18–4.14 (m, 2 H, OCH_2), 3.32–3.19 (m, 3 H, CH_2 + 0.5 × $PhCH_2$), 3.04–3.01 (m, 2 H, CH_2), 2.75 (dd, J_1 = 13.8, J_2 = 9.6 Hz, 1 H, 0.5 × $PhCH_2$) ppm. ^{13}C NMR (150 MHz, $CDCl_3$): δ = 171.9, 153.4, 142.4, 135.0, 129.3, 129.1, 128.9, 127.3, 127.0, 126.6, 122.1 (q, J = 273 Hz, CF_3), 66.2, 55.0, 37.7, 36.7, 29.7, 28.3 (q, J = 40.0 Hz, CNN) ppm. $[\alpha]_D^{23}$ = +48.5 (c = 1, $CHCl_3$). HRMS: calcd. for $C_{21}H_{18}F_3N_3O_3$ $[M + H]^+$ 418.13730; found 418.13743.

***tert*-Butyl (3*R*)-4-[(4*S*)-4-Benzyl-2-oxooxazolidin-3-yl]-4-oxo-3-{4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]benzyl}butanoate (15):** LiHMDS (5.5 mL of a 1 M solution, 5.5 mmol) was put into a flask under an argon atmosphere and cooled to –78 °C. To this was added a solution of compound **14** (2.0 g, 5.0 mmol) in THF (25 mL) over 15 min. The reaction was stirred for 1 h at –78 °C after which *tert*-butyl bromoacetate (2.2 mL, 15 mmol) was added. Then the mixture was slowly warmed to –10 °C in 4 h after which TLC analysis showed a completed reaction. A saturated aq. NH_4Cl solution (50 mL) was added and the mixture was extracted with EtOAc (3 × 30 mL). The combined organic layers were dried with $MgSO_4$ and concentrated under reduced pressure. The resulting crude mixture was purified by column chromatography (10% → 20% EtOAc/petroleum ether) and the product was obtained as a colourless solid (yield: 1.86 g, 3.50 mmol, 70%). ^1H NMR (600 MHz, $CDCl_3$): δ = 7.30–7.25 (m, 4 H, Ph), 7.21–7.18 (m, 3 H, Ph), 7.05 (d, J = 9.0 Hz, 2 H, Ph), 4.55–4.52 (m, 1 H, NCH), 4.42–4.38 (m, 1 H, CH_α), 4.05 (dd, J_1 = 9.0, J_2 = 1.8 Hz, 1 H, 0.5 × OCH_2), 3.92 (t, J = 8.4 Hz, 1 H, 0.5 × OCH_2), 3.23 (dd, J_1 = 13.5, J_2 = 2.7 Hz, 1 H, 0.5 × $PhCH_2$), 3.00 (dd, J_1 = 13.2, J_2 = 6.0 Hz, 1 H, 0.5 × $CH_{2,A}$), 2.79–2.70 (m, 2 H, 0.5 × $PhCH_2$ + 0.5 × $CH_{2,B}$), 2.57 (dd, J_1 = 13.2, J_2 = 9.6 Hz, 1 H, 0.5 × $CH_{2,A}$), 2.25 (dd, J_1 = 16.8, J_2 = 4.2 Hz, 1 H, 0.5 × $CH_{2,B}$), 1.34 (s, 9 H, 3 × CH_3) ppm. ^{13}C NMR (150 MHz, $CDCl_3$): δ = 174.5, 170.8, 152.8, 139.9, 135.3, 129.6, 129.3, 128.7, 127.3, 127.1, 126.4, 121.9 (q, J = 273 Hz, CF_3), 80.6, 65.7, 55.2, 41.0, 37.3, 36.2, 28.1 (q, J = 39.8 Hz, CNN), 27.2 ppm. $[\alpha]_D^{23}$ = +72.8 (c = 1, $CHCl_3$). HRMS: calcd. for $C_{27}H_{28}F_3N_3O_5$ $[M + H]^+$ 532.20538; found 532.20512.

1-Benzyl 4-*tert*-Butyl (2*R*)-2-{4-[3-(Trifluoromethyl)-3*H*-diazirin-3-yl]benzyl}succinate (16): Benzyl alcohol (350 μL, 3.40 mmol) was dissolved in THF (8 mL) and cooled to 0 °C. To this mixture was added *n*BuLi (1.10 mL of a 1.6 M solution, 1.76 mmol) and the reaction was stirred at 0 °C for 30 min. Then a solution of compound **15** (784 mg, 1.47 mmol) in THF (4 mL) was added and the reaction mixture was stirred for 1 h at 0 °C and then warmed to room temperature. After 30 min TLC analysis indicated complete conversion of the starting material. A saturated aq. NH_4Cl solution (20 mL) was added and the mixture was extracted twice with EtOAc (20 mL). The combined organic layers were dried with $MgSO_4$ and concentrated under reduced pressure. After purification of the crude mixture by column chromatography (3% → 10% EtOAc/petroleum ether) the product was obtained as a colourless oil (yield: 558 mg, 1.21 mmol, 82%). ^1H NMR (600 MHz, $CDCl_3$): δ = 7.37 (t, J = 7.2 Hz, 1 H, Ph), 7.33 (d, J = 4.8 Hz, 2 H, Ph), 7.22 (t, J = 3.6 Hz, 2 H, Ph), 7.14 (d, J = 7.8 Hz, 2 H, Ph), 7.05 (d, J = 7.8 Hz, 2 H, Ph), 5.06 (dd, J_1 = 36.6, J_2 = 12.0 Hz, 2 H, $PhCH_2O$), 3.13–3.10 (m, 1 H, CH_α), 2.99 (dd, J_1 = 13.2, J_2 = 7.2 Hz, 1 H, 0.5 × $PhCH_2$), 2.82 (dd, J_1 = 13.8, J_2 = 7.8 Hz, 1 H, 0.5 × $PhCH_2$), 2.60 (dd, J_1 = 16.2, J_2 = 8.4 Hz, 1 H, 0.5 × $CH_{2,\alpha}$), 2.34 (dd, J_1 = 16.8, J_2 = 5.4 Hz, 1 H, 0.5 × $CH_{2,\alpha}$), 1.40 (s, 9 H, 3 × CH_3) ppm. ^{13}C NMR (150 MHz, $CDCl_3$): δ = 173.4, 170.3, 140.0, 135.5, 129.3, 128.1, 128.0, 127.1, 126.4, 126.3, 122.0 (q, J = 273 Hz, CF_3), 80.7, 66.3, 42.8, 37.1, 36.4, 28.1 (q, J = 40.5 Hz, CNN), 27.70 ppm. $[\alpha]_D^{23}$ = +3.6 (c = 1, $CHCl_3$).

(*R*)-4-(Benzyloxy)-4-oxo-3-{4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]-benzyl}butanoic Acid (17): *tert*-Butyl ester **16** (550 mg, 1.19 mmol) was dissolved in DCM (4 mL). To this was added TFA (3 mL) and the reaction was stirred at room temperature. After 1 h TLC analysis indicated a completed reaction. Toluene was added and the mixture was concentrated under reduced pressure. Co-evaporating the mixture twice with toluene resulted in a yellowish oil. This crude product was purified by column chromatography (10% → 60% EtOAc/petroleum ether) and the free carboxylic acid was obtained as a colourless solid (yield: 1.09 g, 3.07 mmol, 97%). ^1H NMR (600 MHz, $CDCl_3$): δ = 11.15 (br. s, 1 H, CO_2H), 7.35 (t, J = 7.2 Hz, 1 H, Ph), 7.32 (d, J = 4.8 Hz, 2 H, Ph), 7.21 (t, J = 3.6 Hz, 2 H, Ph), 7.11 (d, J = 7.8 Hz, 2 H, Ph), 7.04 (d, J = 7.8 Hz, 2 H, Ph), 5.06 (dd, J_1 = 36.6, J_2 = 12.0 Hz, 2 H, $PhCH_2O$), 3.17–3.12 (m, 1 H, CH_α), 3.00 (dd, J_1 = 13.8, J_2 = 7.2 Hz, 1 H, 0.5 × $PhCH_2$), 2.81 (dd, J_1 = 13.8, J_2 = 7.8 Hz, 1 H, 0.5 × $PhCH_2$), 2.74 (dd, J_1 = 17.4, J_2 = 9.0 Hz, 1 H, 0.5 × $CH_{2,\alpha}$), 2.43 (dd, J_1 = 17.4, J_2 = 5.4 Hz, 1 H, 0.5 × $CH_{2,\alpha}$) ppm. ^{13}C NMR (150 MHz, $CDCl_3$): δ = 177.7, 173.4, 139.6, 135.3, 129.4, 128.5, 128.5, 128.4, 127.5, 126.5, 122.0 (q, J = 273 Hz, CF_3), 66.7, 42.4, 37.0, 34.9, 28.2 (q, J = 39 Hz, CNN) ppm.

Benzyl (2*R*)-4-[*tert*-Butoxycarbonyl(*tert*-butyldimethylsilyloxy)-amino]-4-oxo-2-{4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]benzyl}-butanoate (18): Carboxylic acid **17** (71 mg, 0.18 mmol) was dissolved in DCM (1.5 mL) and DMF (1 drop) was added. This mixture was cooled to 0 °C and oxalyl chloride (4 equiv., 0.70 mmol, 60 μL) was added dropwise. The reaction was warmed to room temperature and stirred for 30 min after which gas formation ceased. Toluene was added and the mixture was concentrated in vacuo followed by coevaporation with toluene (2×).

O(TBS)-*N*(Boc)-protected hydroxylamine[44] (1.2 equiv., 0.19 mmol, 48 mg) was dissolved in THF (2 mL) and the solution was cooled to 0 °C. *n*BuLi (1.05 equiv., 0.185 mmol, 0.11 mL of a 1.6 M solution in hexane) was added dropwise and the reaction mixture was stirred at 0 °C for 30 min. In a separate flask the freshly prepared crude acyl chloride was dissolved in THF (2 mL) and cooled to 0 °C. To this the lithiated hydroxylamine mixture was added dropwise and the reaction was stirred at 0 °C for 2.5 h after which TLC analysis indicated complete conversion of compound **17**. A 0.1 M aq. HCl solution (10 mL) was added and the mixture was extracted twice with EtOAc (10 mL). The combined organic layers were extracted with 0.1 M aq. HCl (10 mL) and brine, dried with $MgSO_4$ and concentrated under reduced pressure. The crude mixture was purified by column chromatography (toluene → 3% EtOAc/toluene) and the product was obtained as a colourless oil (yield: 30 mg, 47 μmol, 27%). ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.34–7.31 (m, 3 H, Ph), 7.20 (t, J = 3.6 Hz, 2 H, Ph), 7.15 (d, J = 8.4 Hz, 2 H, Ph), 7.04 (d, J = 7.8 Hz, 2 H, Ph), 5.06–5.01 (m, 2 H, $PhCH_2O$), 3.27–3.21 (m, 2 H, CH_α + 0.5× $PhCH_2$), 2.98 (dd, J_1 = 13.2, J_2 = 6.6 Hz, 1 H, 0.5× $PhCH_2$), 2.90–2.84 (m, 2 H, $CH_{2,\alpha}$), 1.53 (s, 9 H, 3× CH_3), 0.98 (s, 9 H, 3× CH_3), 0.12 (s, 3 H, $SiCH_3$), 0.085 (s, 3 H, $SiCH_3$) ppm. ^{13}C NMR (150 MHz, $CDCl_3$): δ = 174.1, 170.3, 151.8, 140.0, 135.5, 129.4, 128.4, 128.2, 128.1, 127.3, 126.4, 122.1 (q, J = 273 Hz, CF_3), 84.6, 66.4, 42.7, 38.8, 37.4, 28.2 (q, J = 40 Hz, CNN), 27.9, 25.6, 18.0, –5.1, –5.1 ppm.

1-Allyl 4-*tert*-Butyl (2*R*)-2-{4-[3-(Trifluoromethyl)-3*H*-diazirin-3-yl]benzyl}succinate (20): Allyl alcohol (190 μL, 2.75 mmol) was dissolved in THF (5 mL) and cooled to 0 °C. To this mixture was added *n*BuLi (812 μL of a 1.6 M solution, 1.3 mmol) and the reaction was stirred at 0 °C for 30 min. Then a solution of compound **15** (586 mg, 1.10 mmol) in THF (4 mL) was added and the reaction mixture was stirred for 1 h at 0 °C and then warmed to room temperature. After 2.5 h TLC analysis indicated complete conversion of the starting material. A saturated aq. NH_4Cl solution (20 mL) was added and the mixture was extracted twice with EtOAc (20 mL). The combined organic layers were dried with $MgSO_4$ and concentrated under reduced pressure. After purification of the crude mixture by column chromatography (2.5% → 5% EtOAc/petroleum ether) the product was obtained as a colourless oil (yield: 293 mg, 0.71 mmol, 65%). ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.21 (d, J = 8.4 Hz, 2 H, Ph), 7.11 (d, J = 7.8 Hz, 2 H, Ph), 5.83–5.77 (m, 1 H, CH sp^2), 5.22 (d, J = 16.8 Hz, 1 H, 0.5× CH_2 sp^2), 5.18 (d, J = 10.8 Hz, 1 H, 0.5× CH_2 sp^2), 4.57–4.50 (m, 2 H, OCH_2), 3.11–3.07 (m, 1 H, CH_α), 3.02 (dd, J_1 = 13.8, J_2 = 7.2 Hz, 1 H, 0.5× $PhCH_2$), 2.82 (dd, J_1 = 13.8, J_2 = 7.8 Hz, 1 H, 0.5× $PhCH_2$), 2.59 (dd, J_1 = 16.2, J_2 = 8.4 Hz, 1 H, 0.5× $CH_{2,\alpha}$), 2.33 (dd, J_1 = 16.8, J_2 = 5.4 Hz, 1 H, 0.5× $CH_{2,\alpha}$), 1.42 (s, 9 H, 3× CH_3) ppm. ^{13}C NMR (150 MHz, $CDCl_3$): δ = 173.5, 170.6, 140.2, 131.7, 129.5, 127.4, 126.5, 122.1 (q, J = 273 Hz, CF_3), 118.3, 80.9, 65.3, 43.0, 37.2, 36.4, 28.2 (q, J = 40.5 Hz, CNN), 27.9 ppm. $[\alpha]_D^{23}$ = +9.9 (c = 1, $CHCl_3$). HRMS: calcd. for $C_{20}H_{23}F_3N_2O_4$ $[M + H]^+$ 413.16827; found 413.16826.

(2*R*)-4-(Allyloxy)-4-oxo-3-{4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]benzyl}butanoic Acid (21): *tert*-Butyl ester **20** (1.31 g, 3.17 mmol) was dissolved in DCM (10 mL). To this was added TFA (10 mL) and the reaction was stirred at room temperature. After 1 h TLC analysis indicated a completed reaction. Toluene was added and the mixture was concentrated under reduced pressure. Co-evaporating the mixture twice with toluene resulted in yellowish oil. This crude product was purified by column chromatography (10% → 50% EtOAc/petroleum ether) and the free acid was obtained as a colourless solid (yield: 1.09 g, 3.07 mmol, 97%). ^{1}H NMR (600 MHz, $CDCl_3$): δ = 10.80 (br. s, 1 H, CO_2H), 7.20 (d, J = 8.4 Hz, 2 H, Ph), 7.12 (d, J = 8.4 Hz, 2 H, Ph), 5.82–5.76 (m, 1 H, CH sp^2), 5.22 (dd, J_1 = 15.6, J_2 = 1.8 Hz, 1 H, 0.5× CH_2 sp^2), 5.19 (dd, J_1 = 10.8, J_2 = 1.2 Hz, 1 H, 0.5× CH_2 sp^2), 4.57–4.52 (m, 2 H, OCH_2), 3.15–3.10 (m, 1 H, CH_α), 3.05 (dd, J_1 = 13.8, J_2 = 7.2 Hz, 1 H, 0.5× $PhCH_2$), 2.83 (dd, J_1 = 13.8, J_2 = 7.8 Hz, 1 H, 0.5× $PhCH_2$), 2.73 (dd, J_1 = 17.4, J_2 = 9.0 Hz, 1 H, 0.5× $CH_{2,\alpha}$), 2.44 (dd, J_1 = 17.4, J_2 = 5.4 Hz, 1 H, 0.5× $CH_{2,\alpha}$) ppm. ^{13}C NMR (150 MHz, $CDCl_3$): δ = 177.7, 173.3, 139.8, 131.5, 129.5, 127.6, 126.6, 122.0 (q, J = 273 Hz, CF_3), 118.5, 65.5, 42.5, 37.1, 34.8, 28.2 (q, J = 40.0 Hz, CNN) ppm. $[\alpha]_D^{23}$ = +13.2 (c = 1, $CHCl_3$). HRMS: calcd. for $C_{16}H_{15}F_3N_2O_4$ $[M + H]^+$ 357.10567; found 357.10576.

Allyl (2*R*)-4-[*tert*-Butoxycarbonyl(*tert*-butyldimethylsilyloxy)-amino]-4-oxo-2-{4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]benzyl}-butanoate (22): Carboxylic acid **21** (1.22 g, 3.42 mmol) was dissolved in DCM (15 mL) and DMF (2 drops) were added. This mixture was cooled to 0 °C and oxalyl chloride (4 equiv., 13.68 mmol, 1.20 mL) was added dropwise. The reaction was warmed to room temperature and stirred for 30 min after which gas formation ceased. Toluene was added and the mixture was concentrated in vacuo followed by coevaporation with toluene (2×). The crude product was subjected to the next step without further purification. *O*(TBS)-*N*(Boc)-protected hydroxylamine[44] (1.2 equiv., 4.1 mmol, 1.01 g) was dissolved in THF (10 mL) and the solution was cooled to 0 °C. *n*BuLi (1.05 equiv., 3.59 mmol, 2.25 mL of a 1.6 M solution in hexane) was added dropwise and the reaction mixture was stirred at 0 °C for 30 min. In a separate flask the freshly prepared crude acyl chloride was dissolved in THF (15 mL) and cooled to 0 °C. To this the lithiated hydroxylamine mixture was added dropwise and the reaction was stirred at 0 °C for 2.5 h after which TLC analysis indicated complete conversion of compound **21**. A 0.1 M aq. HCl solution (30 mL) was added and the mixture was extracted twice with EtOAc (30 mL). The combined organic layers were extracted with 0.1 M aq. HCl (30 mL) and brine, dried with $MgSO_4$ and concentrated under reduced pressure. The crude mixture was purified by column chromatography (100% toluene → 2.5% EtOAc/toluene) and the product was obtained as a yellowish oil (yield: 1.54 g, 2.63 mmol, 77%). ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.22 (d, J = 8.4 Hz, 2 H, Ph), 7.11 (d, J = 8.4 Hz, 2 H, Ph), 5.80–5.75 (m, 1 H, CH sp^2), 5.21 (d, J = 17.4 Hz, 1 H, 0.5× CH_2 sp^2), 5.16 (d, J = 10.2 Hz, 1 H, 0.5× CH_2 sp^2), 4.51 (d, J = 6.0 Hz, 2 H, OCH_2), 3.24–3.20 (m, 2 H, CH_α + 0.5× $PhCH_2$), 3.03 (dd, J_1 = 13.8, J_2 = 6.6 Hz, 1 H, 0.5× $PhCH_2$), 2.89–2.85 (m, 2 H, $CH_{2,\alpha}$), 1.53 (s, 9 H, 3× CH_3), 0.99 (s, 9 H, 3× CH_3), 0.13 (s, 3 H, $SiCH_3$), 0.10 (s, 3 H, $SiCH_3$) ppm. ^{13}C NMR (150 MHz, $CDCl_3$): δ = 173.7, 170.2, 151.8, 140.3, 131.8, 129.4, 127.3, 126.5, 122.0 (q, J = 273 Hz, CF_3), 118.1, 84.5, 65.2, 42.7, 38.6, 37.3, 28.2 (q, J = 40.5 Hz, CNN), 27.8, 25.6, 18.0, –5.2 ppm. $[\alpha]_D^{23}$ = +7.91 (c = 1, $CHCl_3$). HRMS: calcd. for $C_{27}H_{38}F_3N_3O_6Si$ $[M + H]^+$ 586.25547; found 586.25562.

Pentafluorophenyl (2*R*)-4-[*tert*-Butoxycarbonyl(*tert*-butyldimethylsilyloxy)amino]-4-oxo-2-{4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]-benzyl}butanoate (24): Allyl ester **22** (1.53 g, 2.63 mmol) was dissolved in THF (15 mL). To this solution were added *N*,*N*′-dimethylbarbaturic acid (**23**, 0.5 equiv., 1.32 mmol, 210 mg) and tetrakis(triphenylphosphane)palladium (cat.). The reaction was stirred for 1 h at room temp. after which TLC analysis indicated complete consumption of the starting compound. The mixture was concentrated under reduced pressure and dissolved again in DCM (15 mL) without further purification (**19**). To this were added pentafluorophenol (2 equiv., 5.26 mmol, 556 μL) and EDCI (2 equiv., 5.26 mmol, 1.00 g) and the reaction was stirred for 12 h at room temp. Et_2O (50 mL) was added and the mixture was ex-

tracted twice with 0.1 M aq. HCl (50 mL) and brine, dried with $MgSO_4$ and concentrated under reduced pressure. The resulting mixture was purified by column chromatography (1.5% EtOAc/petroleum ether) and the product was obtained as a yellow solid (yield: 1.65 g, 2.31 mmol, 88%). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.30 (d, J = 8.5 Hz, 2 H, Ph), 7.16 (d, J = 8.0 Hz, 2 H, Ph), 3.62–3.56 (m, 1 H, CH_α), 3.33 (dd, J_1 = 18.5, J_2 = 9.5 Hz, 1 H, 0.5× $PhCH_2$), 2.23 (dd, J_1 = 13.5, J_2 = 6.5 Hz, 1 H, 0.5× $PhCH_2$), 3.07–2.99 (m, 2 H, $CH_{2,\alpha}$), 1.54 (s, 9 H, 3× CH_3), 0.99 (s, 9 H, 3× CH_3), 0.14 (s, 3 H, $SiCH_3$), 0.12 (s, 3 H, $SiCH_3$) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 170.5, 169.6, 151.9, 141.1 (dd, J_1 = 250 H, J_2 = 9.0 Hz, *o*-CF PFP), 139.5 (dt, J_1 = 264, J_2 = 13.5 Hz, *p*-CF_3 PFP), 139.3, 137.8 (dt, J_1 = 249, J_2 = 13.5 Hz, *m*-CF_3 PFP), 129.5, 127.9, 126.8, 125.0 (t, J_2 = 12.8 Hz, C-O PFP), 122.1 (q, J = 273 Hz, CF_3), 84.9, 42.4, 38.7, 37.1, 28.3 (q, J = 40.1 Hz, CNN), 27.8, 25.6, 18.1, –5.2, –5.3 ppm. ^{19}F NMR (188 MHz, $CDCl_3$): δ = –65.8 (s, 3 F), –152.4 (d, J = 18.6 Hz, 2 F), –158.6 (t, J = 21.1 Hz, 1 F), –163.0 (t, J = 21.4 Hz, 2 F) ppm. $[\alpha]_D^{23}$ = +8.8 (c = 1, $CHCl_3$). HRMS: calcd. for $C_{30}H_{33}F_8N_3O_6Si$ [M + Na]$^+$ 734.19031; found 734.19061.

(2*R*)-*N*^{4}-(*tert*-Butoxycarbonyl)-*N*^{4}-(*tert*-butyldimethylsilyloxy)-*N*^{1}-[(2*S*)-1-(methylamino)-1-oxo-3-phenylpropan-2-yl]-2-{4-[3-(trifluoromethyl)-3*H*-diaziren-3-yl]benzyl}succinamide (26): L-phenylalaninemethylamide[43] (**25**, 21 mg, 120 μmol) was added to a solution of compound **24** (85 mg, 120 μmol) in DMF (2 mL). The reaction was stirred at room temp. for 24 h after which no more starting material was consumed (followed by LC-MS analysis). Et_2O (10 mL) and 0.1 M aq. HCl solution (10 mL) were added and the layers were separated. The aq. layer was extracted with Et_2O (10 mL) and the combined organic layers were extracted with a 0.1 M aq. HCl solution (2× 10 mL) and brine, dried with $MgSO_4$ and concentrated under reduced pressure. The resulting mixture was purified by column chromatography (10% → 50% EtOAc/petroleum ether) and the product was obtained as a colourless oil (yield: 38 mg, 54 μmol, 45%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.29–7.17 (m, 7 H, Ph), 7.06 (d, J = 8.0 Hz, 2 H, Ph), 6.14 (d, J = 8.0 Hz, 1 H, NH), 5.29–5.28 (m, 1 H, NH), 4.50 (q, J = 6.9 Hz, 1 H, NCH_α), 3.21–3.11 (m, 2 H, CH_α + 0.5× $PhCH_2$), 2.97–2.85 (m, 4 H, $PhCH_2$ + 0.5× $PhCH_2$ + 0.5× $CH_{2,\alpha}$), 2.77–2.69 (m, 1 H, 0.5× $CH_{2,\alpha}$), 2.57 (d, J = 4.8 Hz, 3 H, NCH_3), 1.54 (s, 9 H, 3× CH_3), 0.99 (s, 9 H, 3× CH_3), 0.14 (s, 3 H, $SiCH_3$), 0.11 (s, 3 H, $SiCH_3$) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 173.6, 170.8, 170.7, 151.8, 140.7, 136.7, 129.5, 128.6, 127.5, 126.9, 126.5, 122.1 (q, J = 273 Hz, CF_3), 84.8, 54.4, 44.5, 39.9, 37.9, 37.6, 29.7, 28.3 (q, J = 40.1 Hz, CNN), 28.0, 26.0, 25.7, 18.1, –4.9 ppm. $[\alpha]_D^{23}$ = +5.6 (c = 1, $CHCl_3$). LC-MS (system A): = gradient 50 → 90 ACN/(0.1 TFA/H_2O): R_t (min): 9.81 [ESI-MS: m/z = 705.87 [M + H$^+$]]. HRMS: calcd. for $C_{34}H_{46}F_3N_5O_6Si$ [M + H]$^+$ 706.32422; found 706.32419.

(2*R*)-*N*^{4}-Hydroxy-*N*^{1}-[(2*S*)-1-(methylamino)-1-oxo-3-phenylpropan-2-yl]-2-{4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]benzyl}succinamide (27): TFA (1 mL) and deionised H_2O (50 μL) were added to a solution of compound **26** (21 mg, 30 μmol) in DCM (1 mL). After 1 h of stirring LC-MS and TLC analysis indicated complete conversion of the starting material. Toluene was added and the mixture was concentrated under reduced pressure. In order to remove excess TFA the mixture was co-evaporated twice with toluene. The resulting crude mixture was purified by HPLC [system B, gradient 40% → 65% ACN/(0.1% TFA/H_2O)]. The title compound was obtained as a colourless solid (yield: 10.2 mg, 21 μmol, 69%). ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 10.44 (s, 1 H, N*H*OH), 8.78 (br. s, 1 H, NHO*H*), 8.17 (d, J = 8.0 Hz, 1 H, NH), 7.65–7.58 (m, 1 H, NH), 7.30–7.21 (m, 7 H, Ph), 7.14 (d, J = 8.0 Hz, 2 H, Ph), 4.38–4.36 (m, 1 H, NCH_α), 3.00 (dd, J_1 = 13.6, J_2 = 5.2 Hz, 1 H, 0.5× $PhCH_2$), 2.98–2.92 (m, 1 H, CH_α), 2.82–2.75 (m, 2 H, 2× 0.5× $PhCH_2$), 2.62 (dd, J_1 = 13.6, J_2 = 5.6 Hz, 1 H, 0.5× $PhCH_2$), 2.57 (d, 3 H, NCH_3), 2.11 (dd, J_1 = 14.8, J_2 = 7.2 Hz, 1 H, 0.5× $CH_{2,\alpha}$), 1.94 (dd, J_1 = 14.6, J_2 = 7.4 Hz, 1 H, 0.5× $CH_{2,\alpha}$) ppm. ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 173.7, 172.0, 168.3, 142.8, 139.0, 130.8, 130.0, 129.0, 127.1, 127.0, 126.2, 55.0, 44.1, 38.2, 37.8, 35.1, 26.4 ppm. $[\alpha]_D^{23}$ = –10.8 (c = 1, DMSO). LC-MS (system A): gradient 10% → 90% ACN/(0.1% TFA/H_2O): R_t (min): 7.20 [ESI-MS: m/z = 492.07 [M + H$^+$]]. HRMS: calcd. for $C_{23}H_{24}F_3N_5O_4$ [M + H]$^+$ 492.18532; found 492.18494.

HA-succ(tmd)-Phe-Ahx-Lys(Biotin)-NH$_2$ (2a): This compound was synthesized on solid support on 20 μmol scale [based on the loading of Fmoc-Lys(Biotin)] following the general procedure for solid-phase synthesis. The final coupling step involved the addition of compound **24** (70 μmol, 50 mg) and DIEA (40 μmol, 90 μL 0.45 M in NMP) in NMP (0.40 mL) to the resin and shaking for 2 h. The compound was purified by HPLC [system B, gradient 10% → 90% ACN/(0.1% TFA/H_2O)] and was obtained as a colourless solid (yield: 4.3 mg, 6.0 μmol, 30% after 3 coupling steps). ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 10.38 (s, 1 H, N*H*OH), 8.25 (br. s, 1 H, NHO*H*), 8.13 (t, J = 8.72, 8.72 Hz, 1 H, NH-Phe), 7.80–7.72 (m, 4 H, NH-Ahx + α-NH-Lys), 7.65 (t, J = 5.58 Hz, 1 H, ε-NH-Lys), 7.37–7.13 (m, 8 H, Ph), 7.10 (t, J = 7.74 Hz, 2 H, Ph), 6.94 (br. s, 1 H, 0.5× NH_2), 6.42 (br. s, 1 H, NH-Biotin), 6.36 (br. s, 1 H, NH-Biotin), 4.35–4.32 (m, 2 H, CH_α-Phe + NCH-Biotin), 4.18–4.09 (m, 2 H, CH_α-Lys + NCH-Biotin), 3.09 (dd, J_1 = 12.83, J_2 = 6.04 Hz, 1 H, SCH), 3.03–2.87 (m, 6 H, 0.5× CH_α + 0.5× $PhCH_2$ + NCH_2-Ahx + NCH_2-Lys), 2.82 (dd, J_1 = 12.40, J_2 = 5.07 Hz, 1 H, 0.5× CH_2S), 2.78–2.72 (m, 1 H, 0.5× CH_α), 2.68–2.66 (m, 1 H, 0.5× $PhCH_2$), 2.64–2.60 (m, 1 H, 0.5× $PhCH_2$), 2.60 (d, J = 12.4 Hz, 1 H, 0.5× CH_2S), 2.13 (t, J = 7.35 Hz, 2 H, $CH_{2,\alpha}$-Ahx), 2.07 (t, J = 7.60 Hz, 2 H, $CH_{2,\alpha}$-Biotin), 2.02–1.96 (m, 1 H, 0.5× $CH_{2,\alpha}$) 1.91 (dd, J_1 = 13.98, J_2 = 7.50 Hz, 1 H, 0.5× $CH_{2,\alpha}$), 1.65–1.56 (m, 1 H, 0.5× CH_2-Lys), 1.52–1.40 (m, 9 H, 0.5× CH_2-Lys + 2× CH_2-Ahx+ 2× CH_2-Biotin), 1.40–1.22 (m, 6 H, 2× CH_2-Lys, CH_2-Biotin), 1.20–1.12 (m, 2 H, CH_2-Ahx) ppm. LC-MS (system A): gradient 10% → 90% ACN/(0.1% TFA/H_2O): R_t (min): 6.45 (ESI-MS: m/z = 945.53 [M + H$^+$]). HRMS: calcd. for $C_{44}H_{59}F_3N_{10}O_8S$ [M + H]$^+$ 945.42629; found 945.42682.

(2*R*)-*N*^{1}-[(2*S*)-1-{6-[(*S*)-1,6-Diamino-1-oxohexan-2-ylamino]-6-oxohexylamino}-1-oxo-3-phenylpropan-2-yl]-*N*^{4}-hydroxy-2-{4-[3-(trifluoromethyl)-3*H*-diazirin-3-yl]benzyl}succinamide or HA-succ(tmd)-Phe-Ahx-Lys-NH$_2$ (30): This compound was synthesized on solid support on 175 μmol scale [based on the loading of Fmoc-Lys(Boc)] following the general procedure for solid-phase synthesis. The final coupling step involved the addition of compound **24** (1.12 mmol, 800 mg) and DIEA (750 μmol, 124 μL 0.45 M in NMP) in NMP (10 mL) to the resin and shaking for 2 h. The compound was purified by HPLC [system B, gradient 10% → 90% ACN/(0.1% TFA/H_2O)] and was obtained as a colourless solid (yield: 45 mg, 54 μmol, 30% after 3 coupling steps). ^{1}H NMR (400 MHz, CD_3OD): δ = 7.69 (t, J = 5.62 Hz, 1 H, NH), 7.38–7.34 (m, 1 H, NH), 7.29–7.16 (m, 9 H, Ph + 2× NH), 7.10 (d, J = 8.03 Hz, 2 H, Ph), 4.43 (t, J = 7.49 Hz, 1 H, NCH_α-Phe), 4.35 (dd, J_1 = 8.89, J_2 = 5.32 Hz, 1 H, NCH_α-Lys), 3.15–3.05 (m, 3 H, NCH_2-Ahx + 0.5× $PhCH_2$-Phe), 3.04–2.99 (m, 1 H, CH_α), 2.98–2.83 (m, 4 H, NCH_2-Lys + 0.5× $PhCH_2$-Phe + 0.5× $PhCH_2$), 2.70 (dd, J_1 = 13.62, J_2 = 6.17 Hz, 1 H, 0.5× $PhCH_2$), 2.33–2.24 (m, 3 H, $CH_{2,\alpha}$-Ahx + 0.5× $CH_{2,\alpha}$), 2.11 (dd, J_1 = 14.82, J_2 = 6.37 Hz, 1 H, 0.5× $CH_{2,\alpha}$), 1.87 (ddd, J_1 = 14.60, J_2 = 7.64, J_3 = 4.70 Hz, 1 H, 0.5× CH_2-Lys), 1.78–1.68 (m, 3 H, 0.5× CH_2-Lys + CH_2-Lys), 1.57 (dd, J_1 = 14.95, J_2 = 7.45 Hz, 2 H, CH_2-Ahx), 1.55–1.45 (m, 2 H, CH_2-

Lys), 1.44–1.36 (m, 2 H, CH_2-Ahx), 1.30–1.18 (m, 2 H, CH_2-Ahx) ppm. ^{13}C NMR (100 MHz, CD_3OD): δ = 177.0, 176.5, 173.8, 173.7, 171.2, 143.4, 139.5, 131.8, 131.3, 130.3, 128.9, 128.6, 128.4, 57.2, 54.9, 46.5, 41.4, 41.0, 39.4, 39.4, 37.4, 36.5, 33.4, 30.7, 28.9, 28.2, 27.2, 24.7 ppm. LC-MS (system A): gradient 10% → 90% ACN/(0.1% TFA/H_2O): R_t (min): 5.92 (ESI-MS: *m*/*z* = 719.27 [M + H^+]). HRMS: calcd. for $C_{34}H_{45}F_3N_8O_6$ [M + H]$^+$ 719.34869; found 719.34848.

HA-succ(tmd)-Phe-Ahx-Lys(Bodipy-Tmr)-NH_2 (2b): Compound **30** (9.1 mg, 11 μmol) and Bodipy-Tmr-OSu[47] (4.6 mg, 12 μmol) were dissolved in DMF (0.5 mL). DIEA (4.5 μL, 27 μmol) was added and the reaction was stirred for 24 h after which the solvent was evaporated under reduced pressure. The resulting mixture was purified by HPLC [system B, gradient 10% → 90% ACN/(0.1% TFA/H_2O)] and the title compound was obtained as a brown solid (yield: 3.4 mg, 3.4 μmol, 31%). ^1H NMR (400 MHz, [D_6]DMSO): δ = 10.38 (s, 1 H, N*H*OH), 8.73 (s, 1 H, NHO*H*), 8.15 (d, *J* = 8.0 Hz, 1 H, NH-Phe), 7.86 (d, *J* = 8.87 Hz, 2 H, Ph-Bodipy), 7.85 (t, *J* = 5.20 Hz, 1 H, NH-Ahx), 7.81 (d, *J* = 8.40 Hz, 1 H, α-NH-Lys) 7.67 (s, 1 H, CH_{ar}-Bodipy), 7.68 (t, *J* = 5.49 Hz, 1 H, ε-NH-Lys), 7.32 (s, 1 H, 0.5× NH_2), 7.30–7.11 (m, 10 H, Ph + CH_{ar}-Bodipy), 7.02 (d, *J* = 8.91 Hz, 2 H, Ph-Bodipy), 6.93 (s, 1 H, 0.5× NH_2), 6.70 (d, *J* = 4.02 Hz, 1 H, CH_{ar}-Bodipy), 4.41–4.30 (m, 1 H, CH_α-Phe), 4.13 (dt, J_1 = 8.80, J_2 = 5.79 Hz, 1 H, CH_α-Lys), 3.82 (s, 3 H, OCH_3), 3.04–2.83 (m, 6 H, NCH_2-Ahx + CH_α + NCH_2-Lys + 0.5× $PhCH_2$-Phe), 2.82–2.70 (m, 2 H, 0.5× $PhCH_2$-Phe + 0.5× $PhCH_2$), 2.64–2.58 (m, 3 H, 0.5× $PhCH_2$ + $PhCH_2$-Bodipy), 2.48 (s, 3 H, CH_3), 2.24 (s, 3 H, CH_3), 2.23 (t, *J* = 6.8 Hz, 2 H, $CH_{2,\alpha}$-Bodipy), 2.14–2.07 (m, 3 H, 0.5× $CH_{2,\alpha}$ + $CH_{2,\alpha}$-Ahx), 1.88 (dd, J_1 = 15.01, J_2 = 7.43 Hz, 1 H, 0.5× $CH_{2,\alpha}$), 1.68–1.58 (m, 1 H, 0.5× CH_2-Lys), 1.54–1.42 (m, 3 H, 0.5× CH_2-Lys + CH_2-Ahx), 1.39–1.11 (m, 8 H, 2× CH_2-Ahx + 2× CH_2-Lys) ppm. ^{19}F NMR (376 MHz, [D_6]DMSO): δ = –64.09 (s, 3 F), –136.82 (q, *J* = 32.8 Hz, 2 F) ppm. LC-MS (system A): gradient 10% → 90% ACN/(0.1% TFA/H_2O): R_t (min): 8.84 (ESI-MS: *m*/*z* = 1099.40 [M + H^+]). HRMS: calcd. for $C_{55}H_{64}BF_5N_{10}O_8$ [M + H]$^+$ 1099.49946; found 1099.50039.

HA-succ(tmd)-Phe-Ahx-Lys(Bodipy-FL)-NH_2 (2c): Compound **30** (9.1 mg, 11 μmol) and Bodipy-FL-OSu[48] (4.6 mg, 12 μmol) were dissolved in DMF (0.5 mL). DIEA (4.5 μL, 27 μmol) was added and the reaction was stirred for 24 h after which the solvent was evaporated under reduced pressure. The resulting mixture was purified by HPLC [system B, gradient 10% → 90% ACN/(0.1% TFA/H_2O)] and the title compound was obtained as an orange solid (yield: 5.9 mg, 6.0 μmol, 55%). ^1H NMR (400 MHz, [D_6]DMSO): δ = 10.38 (s, 1 H, N*H*OH), 8.73 (s, 1 H, NHO*H*), 8.12 (d, *J* = 8.23 Hz, 1 H, NH-Phe), 7.91 (t, *J* = 5.35 Hz, 1 H, NH-Ahx), 7.80 (d, *J* = 8.15 Hz, 1 H, α-NH-Lys), 7.68 (s, 1 H, CH_{ar}-Bodipy), 7.65 (t, *J* = 5.49 Hz, 1 H, ε-NH-Lys), 7.31 (s, 1 H, 0.5× NH_2), 7.27–7.13 (m, 7 H, Ph), 7.11–7.07 (m, 3 H, CH_{ar}-Bodipy + Ph), 6.95 (s, 1 H, 0.5× NH_2), 6.34 (d, *J* = 3.99 Hz, 1 H, CH_{ar}-Bodipy), 6.29 (s, 1 H, CH_{ar}-Bodipy), 4.34 (dd, J_1 = 14.65, J_2 = 8.31 Hz, 1 H, CH_α-Phe), 4.15 (dt, J_1 = 8.29, J_2 = 5.47 Hz, 1 H, CH_α-Lys), 3.15–3.03 (m, 4 H, NCH_2-Ahx + $PhCH_2$-Bodipy), 3.01–2.90 (m, 4 H, CH_α + NCH_2-Lys + 0.5× $PhCH_2$-Phe), 2.83–2.77 (m, 2 H, 0.5× $PhCH_2$-Phe + 0.5× $PhCH_2$), 2.62 (dd, J_1 = 13.6, J_2 = 6.0 Hz, 1 H, + 0.5× $PhCH_2$), 2.50 (s, 3 H, CH_3), 2.49 (t, signal under DMSO signal, 3 H, CH_3-Bodipy) 2.29 (s, 3 H, CH_3-Bodipy), 2.18–2.06 (m, 3 H, 0.5× $CH_{2,\alpha}$ + $CH_{2,\alpha}$-Ahx), 1.88 (dd, J_1 = 15.00, J_2 = 7.51 Hz, 1 H, 0.5× $CH_{2,\alpha}$), 1.70–1.60 (m, 1 H, 0.5× CH_2-Ahx), 1.57–1.38 (m, 5 H, 0.5× CH_2-Ahx + 2× CH_2-Ahx), 1.37–1.23 (m, 4 H, 2× CH_2-Lys), 1.22–1.11 (m, 2 H, CH_2-Ahx) ppm. ^{19}F NMR (376 MHz, [D_6]DMSO): δ = –64.09 (s, 3 F), –142.77 (q, *J* = 33.2 Hz, 2 F) ppm. LC-MS (system A): gradient 10% → 90% ACN/(0.1% TFA/H_2O): R_t (min): 8.13 (ESI-MS: *m*/*z* = 993.33 [M + H^+]). HRMS: calcd. for $C_{48}H_{58}BF_5N_{10}O_7$ [M + H]$^+$ 993.45759; found 993.45864.

Inhibition and Labeling Studies: Recombinant ADAM-8 (Catalog Number 1031-AD, catalytic domain of human ADAM-8 Glu^{158}-Pro^{497}, Murine myeloma cell line, NS0 derived), ADAM-9 (Catalog Number 939-AD, catalytic domain of human ADAM-9 Ala^{206}-Asp^{697}, Murine myeloma cell line, NS0 derived), ADAM-10 [Catalog Number 936-AD, catalytic domain of human ADAM-10 Thr^{214}-Glu^{672}, *Spodoptera frugiperda*, Sf 21 (baculovirus) derived] and ADAM-17 [ectodomain, Catalog Number 930-ADB, catalytic domain of human ADAM-17 Arg^{215}-Asn^{671}, *Spodoptera frugiperda*, Sf 21 (baculovirus) derived] were purchased from R&D systems (Minneapolis, MN, USA). ADAM-8 was autocatalytically activated by incubation at 37 °C for 5 d according to the manufacturer's instructions. Recombinant catalytic domains (CD) of human MMP-1 (Catalog Number BML-SE180–0010, catalytic domain of human MMP-1 Phe^{100}-Gln^{268}), MMP-2 (Catalog Number BML-SE237–0010, catalytic domain of human MMP-2 Tyr^{110}-Asp^{452}), MMP-3 (Catalog Number BML-SE109–0010, catalytic domain of human MMP-3 Phe^{100}-Thr^{272}), MMP-7 (Catalog Number BML-SE181–0010, catalytic domain of human MMP-7 Tyr^{95}-Lys^{267}), MMP-8 (Catalog Number BML-SE255–0010, catalytic domain of human MMP-8 Phe^{99}-Gln^{269}), MMP-10 (Catalog Number BML-SE329–0010, catalytic domain of human MMP-10 Phe^{99}-Glu^{271}), MMP-11 (Catalog Number BML-SE282–0010, catalytic domain of human MMP-11 Phe^{98}-Ser^{266}) and MMP-13 (Catalog Number BML-SE246–0010, catalytic domain of human MMP-13 Tyr^{104}-Asn^{274}) were from Biomol International (Butler Pike, PA, USA). All hrMMPs were expressed in *E. coli*. Recombinant human MMP-12 CD and recombinant human MMP-9 CD without fibronectin type II inserts (expressed in *E. coli* as described[51,52]) were a kind gift from AstraZeneca R&D (Lund & Moelndal, Sweden). TIMP-1 from human neutrophil granulocytes was from Calbiochem (La Jolla, CA, USA). Alkaline phosphatase conjugated streptavidin was from Sigma–Aldrich (Zwijndrecht, The Netherlands). 5-Bromo-4-chloro-3-indoyl phosphate (BCIP) and nitro blue tetrazolium (NBT) were from Duchefa (Haarlem, The Netherlands). Unless mentioned otherwise all other biochemicals were from Sigma–Aldrich.

Determination of IC_{50} Values: The affinity of the photoactivatable probes for ADAM and MMP proteases was determined in a competitive enzyme activity assay monitoring conversion of the fluorogenic substrate Mca-PLAQAV-Dpa-RSSSR-NH_2 (R&D Systems) by recombinant ADAM-9, -10 and -17 in the presence of increasing concentrations photoactivatable probe. For MMP-9 and MMP-12 inhibition of the conversion of fluorogenic substrate Mca-PLGL-Dpa-AR-NH_2 (Bachem, Bubendorf, Switserland) was determined. Measurements were performed in Costar White 96-well plates (Corning, Schiphol-Rijk, The Netherlands), where each well contained either 10 ng ADAM-17, 100 ng ADAM-10 or 200 ng ADAM-9 and a final concentration of 10 μM substrate in a final volume of 100 μL ADAM assay buffer (25 mM Tris pH 9.0, 2.5 μM $ZnCl_2$, 0.005% w/v Brij-35). Inhibition of MMP proteolytic activity was determined with 10 ng of MMP-9 or MMP-12 per well with a final concentration of 2 μM substrate in 100 μL MMP assay buffer [50 mM Tris pH 7.4, 0.2 M NaCl, 10 mM $CaCl_2$, 2.5 μM $ZnCl_2$, 0.05% (v/v) Brij-35]. Proteolysis rates were followed by measuring fluorescence ($\lambda_{ex,em}$ = 320, 440 nm) increase using a Fluostar Optima plate reader (BMG Labtech, Offenburg, Germany) at 37 °C. Six-point inhibition curves (0–10 μM) were plotted in Origin 7.0 (Micronal) and IC_{50} values were determined by sigmoidal fitting.

Labeling of Active Recombinant Metalloproteases: Recombinant MMP catalytic domains and recombinant ADAM ectodomains were incubated with photoactivatable inhibitor probes in 96-well plates (Costar White). Each well (final volume 30 μL) contained 4 pmol enzyme and a final concentration of 1 μM inhibitor probe in MMP or ADAM assay buffer. The plate was irradiated at 366 nm using a Camag universal UV lamp (20 W, distance to plate 4 cm) for 30 min. For subsequent analysis by Western blotting, the reaction was stopped by adding 10 μL 5× non-reducing SDS-PAGE sample buffer.

Western Blotting: Samples were analyzed by SDS-PAGE on 0.75 mm thick 12.5% polyacrylamide gels. Electrophoresis was carried out at 20 mA per gel using a mini*i*Protean III electrophoresis system (Bio-Rad, Veenendal, The Netherlands). The proteins were transferred to an Immun-Blot PVDF membrane by wet Western blotting in a mini Trans-blot cell at 350 mA for 60 min in 25 mM Tris, 190 mM glycine with 20% (v/v) methanol (BioRad). Membranes were blocked overnight at 4 °C in TBST [25 mM Tris buffer pH 7.5 containing 150 mM NaCl, 0.05% (v/v) Tween-20] supplemented with 5% (w/v) non-fat dried milk (Protifar Plus, Nutricia, Zoetermeer, The Netherlands) and incubated for 1 h in a 1:1500 dilution of streptavidin-alkaline phosphatase (0.67 μg/mL) in TBST supplemented with 1% non-fat dried milk. Biotinylated proteins were visualized by staining with an NBT/BCIP substrate solution (0.1 M Tris buffer, pH 9.5 containing 5 mM $MgCl_2$, 0.15 mg/mL BCIP and 0.30 mg/mL NBT).

Competition with TIMPs: Aliquots of 4 pmol of MMP-9 and MMP-12 were incubated overnight with equimolar equivalents of TIMP-1. Control aliquots were kept at 4 °C overnight without TIMPs. Photoactivatable inhibitor **2a** was added to a final concentration of 200 nM. Labeling and analysis were performed as described above.

Competition with 27: Aliquots of 4 pmol MMP-9 and MMP-12 (both catalytic domains) in assay buffer were preincubated for 15 min with 400 nM control inhibitor **27** and irradiated with UV light. Positive controls were treated the same, but without control inhibitor **27** added to the solution. Next, photoactivatable probe **2a** was added to a final concentration of 200 nM. Labeling and analysis were performed as above.

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[1] J. M. White, *Curr. Opin. Cell Biol.* **2003**, *15*, 598–606.
[2] D. F. Seals, S. A. Coutneidge, *Genes Dev.* **2003**, *17*, 7–30.
[3] C. Chang, Z. Werb, *Trends Cell Biol.* **2001**, *11*, 27–43.
[4] H. D. Foda, S. Zucker, *Drug Discovery Today* **2001**, *6*, 478–482.
[5] M. D. Sternlicht, Z. Werb, *Annu. Rev. Cell Dev. Biol.* **2001**, *17*, 463–516.
[6] K. Iba, R. Albrechtsen, B. Gilpin, C. Frohlich, F. Loechel, A. Zolwiewska, K. Ishiguro, T. Kojima, W. Liu, J. K. Langford, R. D. Sanderson, C. Brakebusch, R. Fassler, U. M. Wewer, *J. Cell Biol.* **2000**, *149*, 1143–1156.
[7] J. Brynskov, P. Foegh, G. Petersen, C. Ellervik, T. Kirkegaard, A. Bingham, T. Saermark, *Gut* **2002**, *51*, 37–43.
[8] R. A. Black, C. T. Rauch, C. J. Kozlosky, J. J. Peschon, J. L. Slack, M. F. Wolfson, B. J. Castner, K. L. Stocking, P. Reddy, S. Srinivasan, N. Nelson, N. Boiani, K. A. Schooley, M. Gerhart, R. Davis, J. N. Fritzner, R. S. Johnson, R. J. Paxton, C. J. March, D. P. Ceretti, *Nature* **1997**, *385*, 729–733.
[9] T. Umata, M. Hirata, T. Takahashi, F. Ryu, S. Shida, Y. Takahashi, M. Tsuenoka, Y. Miura, M. Masuda, Y. Horiguchi, E. Mekada, *J. Biol. Chem.* **2001**, *276*, 30475–30482.
[10] S. Lammich, E. Kojro, R. Postina, S. Gilbert, R. Pfeiffer, M. Jasionowski, C. Haass, F. Fahrenholz, *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 3922–3927.
[11] E. Six, D. Ndiaye, Y. Laabi, C. Brou, N. Gupta-Rossi, A. Israel, F. Logeat, *Proc. Natl. Acad. Sci. USA* **1999**, *100*, 7638–7643.
[12] M. DiMartino, C. Wolff, W. High, G. Stroup, S. Hoffman, J. Laydon, J. C. Lee, D. Bertolini, W. A. Galloway, M. J. Crimmin, M. Davis, S. Davies, *Inflamm. Res.* **1997**, *46*, 211–215.
[13] D. I. Dominguez, B. De Strooper, W. Annaert, *Amyloid* **2001**, *8*, 124–142.
[14] A. H. Drummond, P. Beckett, P. D. Brown, E. A. Bone, A. H. Davidson, W. A. Galloway, A. J. Gearing, P. Huxley, D. Laber, M. McCourt, M. Whittaker, L. M. Wood, A. Wright, *Ann. N. Y. Acad. Sci.* **1999**, *878*, 228–235.
[15] G. Murphy, *Nat. Rev. Cancer* **2008**, *8*, 929–941.
[16] P. W. Fedak, D. S. Smookler, Z. Kassiri, N. Ohno, K. J. Leco, S. Verma, D. A. Mickle, K. L. Watson, C. V. Hojilla, W. Cruz, R. D. Weisel, R. K. Li, R. Khokha, *Circulation* **2004**, *10*, 2401–2409.
[17] T. Klein, P. Geurink, H. S. Overkleeft, K. Kauffman, R. Bischoff, *ChemMedChem* **2009**, *4*, 164–170.
[18] J. H. Vernooy, J. H. Lindeman, J. A. Jacobs, R. Hanemaaijer, E. F. Wouters, *Chest* **2004**, *126*, 1802–1810.
[19] M. Fonovic, S. H. L. Verhelst, M. T. Sorum, M. Bogyo, *Mol. Cell Proteomics* **2007**, *6*, 1761–1770.
[20] Y. Yasuda, Z. Li, D. Greenbaum, M. Bogyo, E. Weber, D. Bromme, *J. Biol. Chem.* **2004**, *279*, 36761–36770.
[21] D. Greenbaum, A. Baruch, L. Hayrpetian, Z. Darula, A. Burlingame, K. F. Medzihradszky, M. Bogyo, *Mol. Cell Proteomics* **2002**, *1*, 60–68.
[22] M. Bogyo, S. Verhelst, V. Bellingard-Dubouchaud, S. Toba, D. Greenbaum, *Chem. Biol.* **2000**, *7*, 27–38.
[23] Y. S. Liu, M. P. Patricelli, B. F. Cravatt, *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 14694–14699.
[24] D. Kidd, Y. Liu, B. F. Cravatt, *Biochemistry* **2001**, *40*, 4005–4015.
[25] G. C. Adam, E. J. Sorensen, B. F. Cravatt, *Mol. Cell Proteomics* **2002**, *1*, 781–790.
[26] A. Tuin, H. Overkleeft, G. van der Marel, A. Fidder, R. van den Berg, M. Mol, D. Noort, *Chem. Res. Toxicol.* **2009**, *22*, 683–689.
[27] M. Verdoes, C. R. Berkers, B. I. Florea, P. F. Van Swieten, H. S. Overkleeft, H. Ovaa, *Methods Mol. Biol.* **2006**, *328*, 51–69.
[28] A. M. Lennon-Duménil, A. H. Bakker, R. Maehr, E. Fiebiger, H. S. Overkleeft, M. Rosemblatt, H. L. Ploegh, C. Lagaudriere-Gesbert, *J. Exp. Med.* **2002**, *196*, 529–540.
[29] B. M. Kessler, D. Tortorella, M. Altun, A. F. Kisselev, E. Fiebiger, B. G. Hekking, H. L. Ploegh, H. S. Overkleeft, *Chem. Biol.* **2001**, *8*, 913–929.
[30] For recent work about photoaffinity labeling of metalloproteases see: a) S. A. Sieber, S. Niessen, H. S. Hoover, B. F. Cravatt, *Nat. Chem. Biol.* **2006**, *2*, 274–281; b) A. Saghatelian, N. Jessani, A. Joseph, M. Humphrey, B. F. Cravatt, *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 10000–10005; c) S. Bregant, C. Huillet, L. Devel, A. S. Dabert-Gay, F. Beau, R. Thai, B. Czarny, A. Yiotakis, V. Dive, *J. Proteome Res.* **2009**, *8*, 2484–2492; d) A. S. Dabert-Gay, B. Czarny, E. Lajeunesse, R. Thai, H. Nagase, V. Dive, *Bioconjugate Chem.* **2009**, *20*, 367–375; A. S. Dabert-Gay, B. Czarny, L. Devel, F. Beau, E. Lajeunesse, S. Bregant, R. Thai, A. Yiotakis, V. Dive, *J. Biol. Chem.* **2008**, *283*, 31058–31067; e) A. David, D. Steer, S. Bregant, L. Devel, A. Makaritis, F. Beau, A. Yiotakis, V. Dive, *Angew. Chem. Int. Ed.* **2007**, *46*, 3275–3277; f) E. W. Chan, S. Chattopadhaya, R. C. Panicker, X. Huang, S. Q. Yao, *J. Am. Chem. Soc.* **2004**, *126*, 14435–14446.

[31] For recent work about the synthesis of ABPs for MMPs, see: a) M. Uttamchandani, C. H. S. Lu, S. Q. Yao, *Acc. Chem. Res.* **2009**, *42*, 1183–1192; b) J. Wang, M. Uttamchandani, J. Li, M. Hu, S. Q. Yao, *Chem. Commun.* **2006**, 3783–3785.
[32] M. A. Leeuwenburgh, P. P. Geurink, T. Klein, H. F. Kauffman, G. A. van der Marel, R. Bischoff, H. S. Overkleeft, *Org. Lett.* **2006**, *8*, 1705–1708.
[33] K. Makos, *Biochimie* **2005**, *87*, 249–263.
[34] H. Matter, M. Schudok, *Curr. Opin. Drug Discov. Devel.* **2004**, *7*, 513–535.
[35] T. S. Rush III, R. Powers, *Curr. Opin. Med. Chem.* **2004**, *4*, 1311–1327.
[36] K. Makos, W. Bode, *Mol. Biotechnol.* **2003**, *25*, 241–266.
[37] P. Geurink, T. Klein, M. Leeuwenburgh, G. van der Marel, H. Kauffman, R. Bischoff, H. Overkleeft, *Org. Biomol. Chem.* **2008**, *6*, 1244–1250.
[38] The route of synthesis we employed is essentially the same as reported, however in this work no characterisation of the final product and intermediates leading to this are given. See: K. Hashimoto, T. Yoshioka, C. Morita, M. Sakai, T. Okuna, H. Shirahama, *Chem. Lett.* **1998**, *3*, 302–304.
[39] Prepared according to the procedure described for its enantiomer: H. Matsunaga, T. Ishizuka, T. Kunieda, *Tetrahedron* **1997**, *53*, 1275–1294.
[40] J. M. Altenburger, C. Mioskowski, H. d'Orchymont, D. Schirlin, C. Schalk, C. Tarnus, *Tetrahedron Lett.* **1992**, *33*, 5055–5058.
[41] In a similar procedure both the methyl and trichloroethyl esters were readily prepared. However these proved less suitable precursors towards the preparation to key intermediate **24**.
[42] a) Y. Wu, Y.-P. Sun, Y.-Q. Yang, Q. Hu, Q. Zhang, *J. Org. Chem.* **2004**, *69*, 6141–6144; b) J. Patel, G. Clavé, P.-Y. Renard, X. Franck, *Angew. Chem. Int. Ed.* **2008**, *47*, 4224–4227.
[43] D. Zhang, X. Xing, G. D. Cuny, *J. Org. Chem.* **2006**, *71*, 1750–1753.
[44] Compound **27** is a close analogue of a reported potent and broad-spectrum metalloprotease inhibitor featuring the leucine side-chain grafted onto the succinyl hydroxamate. See: D. E. Levy, F. Lapierre, W. Liang, W. Ye, C. W. Lange, X. Li, D. Grobelny, M. Casabonne, D. Tyrrell, K. Holme, A. Nadzan, R. E. Galardy, *J. Med. Chem.* **1998**, *41*, 199–223.
[45] Bodipy-Tmr-OSu was prepared using our modified procedure of the synthesis described in the patents for related compounds: a) H. C. Kang, R. P. Haugland, December **1993**, U. S. patent 5,274,113; b) H. C. Kang, R. P. Haugland, September **1995**, U. S. patent 5,451,663; c) R. P. Haugland, H. C. Kang, September **1988**, U. S. patent 4,774,339. See for our reported protocol: ; d) M. Verdoes, B. I. Florea, V. Menendez-Benito, C. J. Maynard, M. D. Witte, W. A. van der Linden, A. M. C. H. van den Nieuwendijk, T. Hofmann, C. R. Berkers, F. W. B. van Leeuwen, T. A. Groothuis, M. A. Leeuwenburgh, H. Ovaa, J. J. Neefjes, D. V. Filippov, G. A. van der Marel, N. P. Dantuma, H. S. Overkleeft, *Chem. Biol.* **2006**, *13*, 1217–1226.
[46] Bodipy-FL-OSu was prepared in a procedure analogous to that for Bodipy-Tmr-OSu.
[47] M. Hashimoto, Y. Hatanaka, *Eur. J. Org. Chem.* **2008**, 2513–2523.
[48] D. D. Cataldo, M. M. Gueders, N. Rocks, N. E. Sounni, B. Evrard, P. Bartsch, R. Louis, A. Noel, J. M. Foldart, *Cell Mol. Biol.* **2003**, *49*, 875–884.
[49] Y. Kobayashi, Y. Tokoro, K. Watatani, *Eur. J. Org. Chem.* **2000**, *23*, 3825–3834.
[50] D. O. Kiesewetter, *Tetrahedron: Asymmetry* **1993**, *4*, 2183–2198.
[51] A. A. Parkar, M. D. Stow, K. Smith, A. K. Panicker, J. P. Guilloteau, R. Jupp, S. J. Crowe, *Protein Expr. Purif.* **2000**, *20*, 152–161.
[52] J. M. Shipley, G. A. Doyle, C. J. Fliszar, Q. Z. Ye, L. L. Johnson, S. D. Shapiro, H. G. Welgus, *J. Biol. Chem.* **1996**, *271*, 4335–4341.

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