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Design and Synthesis of α-Ketoamides as Cathepsin S Inhibitors with Potential Applications against Tumor Invasion and Angiogenesis

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A series of small molecules bearing an α-ketoamide warhead were synthesized and evaluated for their ability to inhibit cathepsin S, a key proteolytic enzyme upregulated in many cancers during tumor progression and metastasis. Most of the synthetic compounds were noncytotoxic, but several robustly inhibited cathepsin S (IC_{50} < 10 nM) and potently suppressed cell migration, invasion, and capillary tube formation. These results highlight the potential of α-ketoamide therapy for preventing or delaying cancer spread.

Introduction

For most cancer patients, tumor invasion and distant metastasis are the major limitations of successful treatment.[1] It is therefore important to develop effective strategies to delay, if not prevent, malignant cells from spreading and forming secondary metastatic colonies. Cathepsin S, also known as Cat S or CTSS,[a] is a cellular protease required for invasion and angiogenesis of cancers.[2,3] This proteolytic enzyme is localized primarily in lysosomes and participates in the generation of class II major histocompatibility complex (MHC)−antigenic peptide complexes.[4] It has been suggested that Cat S can also be translocated to the cell surface or secreted into the extracellular milieu to help degrade extracellular matrix (ECM) components.[5] Unlike other members of the cathepsin family, Cat S is unique because it is highly active and stable at neutral pH,[6] properties compatible with its distinct role in mediating matrix degradation and cell invasion. Consistent with these characteristics, an increase in Cat S expression is often associated with tumor progression and adverse outcome.[7,8] Recently, a Cat S specific antibody termed Fsn0503 has been generated and shown not only to inhibit tumor cell invasion but also to block endothelial cell capillary tube formation.[9] These studies indicate that Cat S may be a prognostic indicator and a molecular target for anticancer drug development.[2,3,9,10]

As a cysteine protease, Cat S contains a catalytic triad formed by the unpaired Cys25 residue along with residues His164 and Asn184.[11] The active form of Cat S cleaves target substrates through nucleophilic attack by the thiolate anion of Cys25 (forming an ion pair with His164) to the carbonyl carbon of the peptide bond followed by hydrolysis of the resulting thioester.[11] On the basis of this proteolytic mechanism, a variety of peptide analogue inhibitors have been designed to irreversibly[12,13] or reversibly[14] inactivate Cat S. Although most irreversible inhibitors, such as low-selective LHVS (*N*-morpholinurea-leucine-homophenyl-alanine-vinylsulfone-phenyl)[12] or E-64 (*trans*-epoxy succinyl-L-leucylamido-(4-guanidino)butane),[13] form covalent complexes with Cat S to inactivate the enzyme, they may trigger undesired immune responses and side effects. In contrast, some reversible inhibitors show less toxicity, but their inhibitory activities against Cat S are not as substantial. Here, we report the design and synthesis of a new class of noncytotoxic α-ketoamides as potent Cat S inhibitors. These compounds not only robustly inhibit Cat S but also exert profound anti-invasive effects on CL1-5 cells of lung adenocarcinoma (the most common form of lung cancer) and antiangiogenic activities against HUVEC cells (the representative model of endothelial cells undergoing angiogenesis).

Results and Discussion

Chemistry. As shown in Figure 1, a known cyano compound, **1**, was previously demonstrated to inhibit Cat S with an IC_{50} of 6 nM.[14] Recently, several ketone-based inhibitors have been identified as promising electrophilic warhead candidates with oral bioavailability.[15] Prompted by these observations, we designed and optimized a series of Cat S inhibitors derived from compound **1**; the P1 and P2 substituents were retained and the cyano warhead was replaced with an α-ketoamide (Figure 1). To increase the affinity between the synthetic inhibitor and Cat S, various P3 substituents were incorporated by coupling with different acids. Initially, the serine-based aldehyde **2** was synthesized from Boc-Ser-OH (Scheme 1). This compound then underwent

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[a] Abbreviations: Cat S or CTSS, cathepsin S; Cat L, cathepsin L; Cat B, cathepsin B; MHC, major histocompatibility complex; HUVEC, human umbilical vein endothelial cells; ECM, extracellular matrix; DMSO, dimethyl sulfoxide; EDC, 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide; TEMPO, 2,2,6,6-tetramethylpiperidine-1-oxyl; MTT, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide; AMC, 7-amino-4-methyl coumarin.

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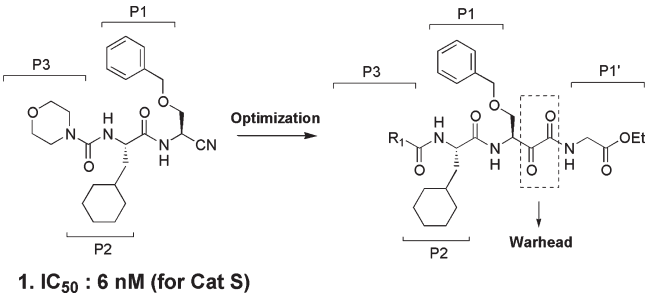
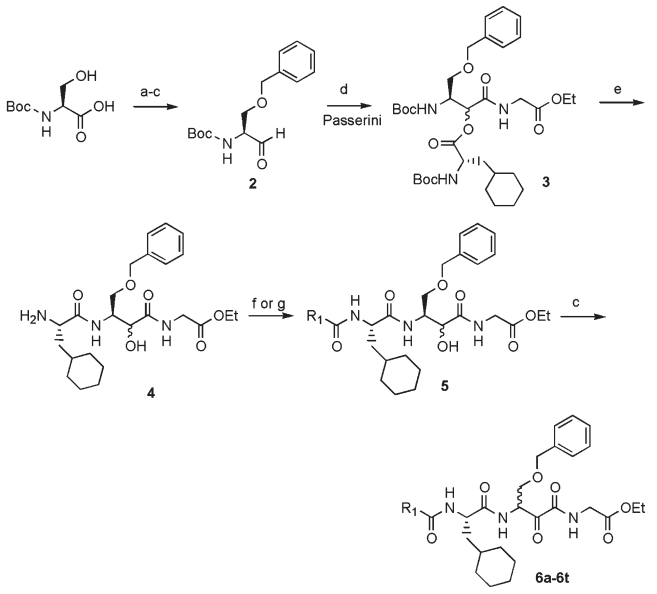


Figure 1. Proposed structural optimization of compound **1**, a cyano-based inhibitor of Cat S. The dashed box (right) represents the ketone-based warhead.

Scheme 1. Synthesis of α-Ketoamide Inhibitors[a]



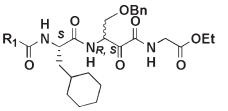
[a] Reagents and conditions: (a) BnBr, NaH, DMF, 0 °C; (b) *N*-methylmorpholine, isobutyl chloroformate, CH_2Cl_2, −20 °C, then $NaBH_4$, H_2O, 0 °C (80% yield for two steps); (c) Dess−Martin periodinane, CH_2Cl_2, rt (96%); (d) Boc-Cha-OH, ethyl isocyanoacetate, CH_2Cl_2, rt (75%); (e) TFA, CH_2Cl_2, rt, then Et_3N, rt (62%); (f) 4-morpholinecarbonyl chloride, Et_3N, DMF, rt (82%); (g) R_1COOH (789 different acids were used), HBTU, Et_3N, DMF. The Dess-Martin oxidation of hydroxyl amide intermediates gave α-ketoamide inhibitors as a mixture of diastereomers at the α-center of the P1 site,[19,20] with diastereomeric ratios being 2:1 to 1:1 (*S*,*S*:*R*,*S*).

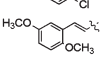
the key multicomponent Passerini reaction[16] with *N*-Boc-protected cyclohexyl alanine and ethyl isocyanoacetate to yield compound **3**. Acidic deprotection of the *N*-Boc moieties on **3** was followed by *O*- to *N*-acyl group migration to generate the α-hydroxyl dipeptide **4** in high yield by adjusting the solution pH to 9.0 with triethylamine.[17] The α-ketoamide precursor **4** was then coupled with 789 different acids to generate a library of α-hydroxyl compounds (**5**). Some of the selected library compounds were subsequently oxidized using Dess−Martin reagent to yield corresponding α-ketoamides **6a**−**6t** (Scheme 1).

Previous studies have suggested that α-hydroxyl amides may be protease inhibitors[18] even though they are less effective inhibitors than the corresponding α-ketoamides. Thus, to facilitate the identification and development of potent Cat S inhibitors, the crude α-hydroxyl amides were subjected to 96-well ELISA-based assays (Supporting Information (SI) Figure S1) to quantify their effects on the activity of Cat S. Only those compounds that inhibited Cat S activity by more than 30% at 100 μM were selected as target compounds. Of a total 789 crude α-hydroxyl amides screened using this method, 30 compounds were found to exhibit significant inhibitory activity against Cat S. These molecules were then resynthesized and purified as pure α-ketoamides and the IC_{50} values were determined in vitro (Table 1 and SI Table S1).

During the synthesis of derivatives with a hydroxyl or amino group at the P3 position, we observed that the yields of desired products were low in the last oxidation step using Dess−Martin reagent. Alternative attempts using mild

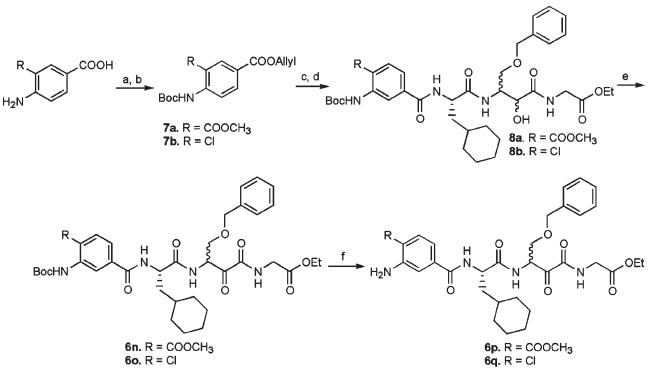
Table 1. Inhibition of Cat S Enzymatic Activity and Cellular Migration by α-Ketoamide-Based Inhibitors



Compd	R1	DR (*S*,*S* : *R*,*S*)[a]	IC_{50} (nM)	Cellular evaluation (Migration ability, % control)	
				CL1-5 cells	HUVEC cells
Control (DMSO)[b]		–	–	100	100
1			5.5	67 ± 13	83 ± 15
6a		52:48	4.4	51 ± 19	87 ± 13
6b		92:8	1.9	67 ± 6	39 ± 4
6c		79:21	8.0	76 ± 10	68 ± 5
6d		81:19	22.8	80 ± 3	73 ± 10
6e		67:33	3.1	95 ± 6	98 ± 0
6f		61:39	2.0	69 ± 5	27 ± 15
6g		57:43	3.6	65 ± 21	N.D.[c]
6h		62:38	3.8	81 ± 9	59 ± 14
6i		100:0	6.6	56 ± 9	57 ± 10
6j		59:41	7.2	67 ± 10	59 ± 14
6k		77:23	10.3	64 ± 11	56 ± 8
6l		52:48	3.4	101 ± 6	95 ± 7
6m		70:30	4.3	98 ± 4	89 ± 1
6n		100:0	49.2	51 ± 10	82 ± 14
6o		69:31	15.7	50 ± 8	48 ± 7
6p		72:28	5.8	53 ± 12	49 ± 10
6q		73:27	2.1	68 ± 12	46 ± 9
6r[d]		100:0	8.8	50 ± 18	55 ± 10
6s		85:15	>100	N.D.[e]	N.D.[e]
6t		68:32	>100	N.D.[e]	N.D.[e]

[a] DR = Diastereomeric ratio of the compounds was determined by ^1H NMR spectrometry in $CDCl_3$. [b] The DMSO control group represents cells (2×10^5) treated with a mixture of DMSO (10 μL) and medium (2 mL) per assay well. [c] HUVEC cells are very sensitive to **6g** in low serum condition, thus the antimigration effect of **6g** was not evaluated. [d] The P2 of **6r** is L-leucine. [e] N.D.: not determined.

Scheme 2. Synthesis of α-Ketoamide Inhibitors with Amino Group at P3 Site[a]



[a] Reagents and conditions: (a) allyl bromide, Cs_2CO_3, DMF, rt (95%); (b) $(Boc)_2O$, MeOH, rt (25%); (c) $Pd(PPh_3)_4$, MeNHPh. THF, rt (90%); (d) **4**, HBTU, Et_3N, DMF, rt (83%); (e) Dess−Martin periodinane, CH_2Cl_2, rt (78%); (f) TFA, CH_2Cl_2, rt (86%).

oxidation reagents such as 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC)/dichloroacetic acid, EDC/pyridinium trifluoroacetate, or 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) also resulted in low yields (i.e., less than 5%, 27%, and 15%, respectively). This problem was finally circumvented by protection of the amino (or hydroxyl) group at P3 before oxidation (Scheme 2). For instance, in the synthesis of compounds **6p** and **6q**, the carboxylic acid moiety of aminobenzoic acid was protected as an allyl ester followed by amine protection using di-*tert*-butyldicarbonate (Boc_2O). However, the low reaction rate between the poorly nucleophilic aromatic amine and Boc_2O resulted in a low yield. The yield of the protected product was improved by adding protic solvents such as methanol[21] to give **7a** and **7b**. Removal of the allyl group was followed by coupling with core structure **4** to generate compounds **8a** and **8b**, which were oxidized and then deprotected under acidic conditions to produce α-ketoamides **6p** and **6q**. Notably, compounds **6s** and **6t**, which do not possess inhibitory activity against Cat S, were synthesized with intent to serve as the negative control compounds in the subsequent bioassays.

Bioassays. The inhibition assays of human recombinant Cat S, which was expressed in *Escherichia coli* (SI Figure S2), were performed mainly as described previously.[22] A total of 22 α-ketoamides were identified to inhibit Cat S with IC_{50} values less than 10 nM (Table 1 and SI Table S1), indicating a successful screening strategy. Unlike cyano inhibitor **1**, which inhibits primarily against Cat S, some of the synthetic α-ketoamides (i.e., **6a**, **6b**, **6f**, **6n**, and **6p**) were found to exert strong cross-inhibitory activity (at 40 nM) against cathepsin L (SI Figure S3). Compound **6p** was further revealed to partially inactivate cathepsin B but required a higher concentration (100 nM) for inhibition (SI Figure S3). These additional inhibitory activities may provide extra benefit for the developed α-ketoamides as anticancer agents due to the involvement of cathepsins L and B in malignant progression.[4] Notably, the presence of amino group at the P3 site shows the enhancement of activity (**6p** and **6q** vs **6n** and **6o**). On the basis of the IC_{50} values listed in Table 1, those efficient compounds were subjected to cell-based assays to evaluate anti-invasive and antiangiogenic activities. Human umbilical vein endothelial cells (HUVEC) and high-invasive CL1-5 lung cancer cells, both of which express high levels of Cat S, were used for the cell-based bioassays. MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide) proliferation assay was carried

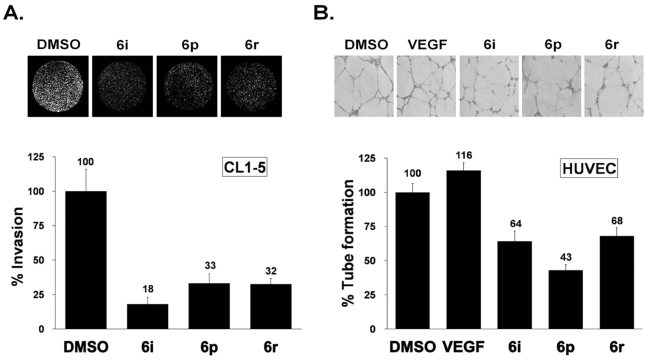


Figure 2. Comparison of inhibition of cell invasion and tube formation by α-ketoamides **6i**, **6p**, and **6r**. (A) Human high-invasive CL1-5 lung cancer cells were applied for the in vitro invasion assay using the FluoroBlok invasion system (www.bdbiosciences.com) to examine the anti-invasive effects of the synthetic molecules. The results show that, in comparison with the invasiveness of control cells in dimethyl sulfoxide (DMSO) set as 100%, the addition of compounds **6i**, **6p**, and **6r** (schematically represented on top) resulted in a significant decrease of cell invasiveness by 82%, 67%, and 68%, respectively (lower panel). Bar graphs reflect the mean percent CL1-5 cell traversal and invasion relative to the control DMSO group ± standard deviation (SD) from hexaplicate experiments. (B) In vitro HUVEC capillary tube formation assays were performed using 96-well plates coated with BD Matrigel. The cells were treated with α-ketoamides in the presence of VEGF (20 ng/mL). Cultures were photographed after 16 h of incubation and capillary tube formation was determined by counting under microscope (original magnification 100×). While VEGF alone was shown to stimulate the branch point formation (top panel), the addition of α-ketoamides **6i**, **6p**, and **6r** resulted in a substantial reduction of VEGF-induced capillary-like tube formation by 36%, 57%, and 32%, respectively (lower panel). Data reflect mean tube formation relative to the control (DMSO) group ± standard deviation (SD) from three separate experiments.

out to examine the overall cytotoxicity of these peptidyl α-ketoamides. Most compounds had no obvious cytotoxicity at concentrations as high as 50 μM (SI Table S2). As summarized in Table 1, some of the α-ketoamides inhibited cell migration to varying degrees. It is of interest to note that few compounds with the lowest IC_{50} values (i.e., **6e**, **6l**, and **6m**) do not correlate with their potency in inhibiting cell migration. This may be best elucidated by their rapid decomposition during the cell-based assays (SI Figure S4a−d). Compounds **6i**, **6p**, and **6r** had an $IC_{50} < 10$ nM and exhibited the greatest inhibition of both CL1-5 and HUVEC cell migrations (~50%). The promising bioactivities of these inhibitors may be explained by the small molecule inhibitors that do not enter cells but have a profound effect by targeting cell-surface or secreted cysteine cathepsins. Because of leaving the intracellular cysteine cathepsins inside cells untouched, the toxicity of inhibitor was minimized. Therefore, these three compounds were further analyzed for their ability to inhibit CL1-5 invasiveness and HUVEC angiogenic tube formation. As shown in Figure 2, all three compounds were capable of significantly suppressing the invasive and angiogenic properties of the test cells, with compound **6p** being the most suppressive. In addition, results from ECM degradation assays revealed that these molecules can repress the cleavage of fibronectin protein by Cat S in a dose-dependent manner, thus suggesting a direct interaction of the α-ketoamides with Cat S that, subsequently, leads to decreased extracellular fibronectin degradation and tumor cell invasion (unpublished observation).

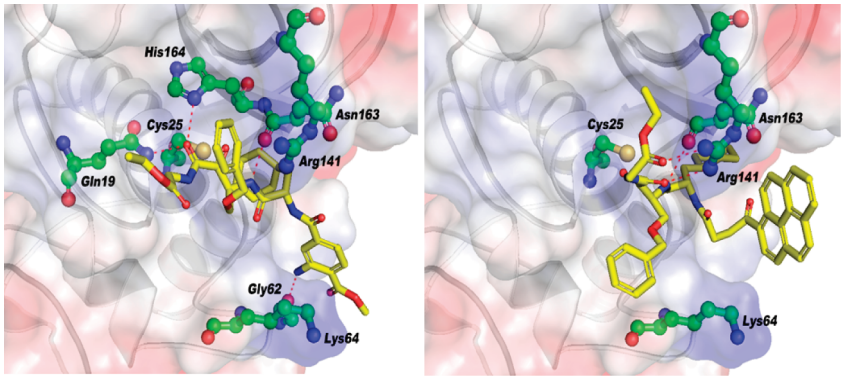


Figure 3. Models of Cat S-inhibitor complexes generated using the LigandFit docking program. The interaction between **6p** and Cat S is illustrated at left and the interaction between **6t** with Cat S is illustrated at right. Red dashed lines indicate hydrogen bonds.

To evaluate the Cat S binding characteristics of the α-ketoamide inhibitors generated in this study, the LigandFit docking program[23] was used to dock various inhibitors with Cat S (SI Figure S5). Two complex models, one with compound **6p**, a robust Cat S inhibitor, and another with compound **6t**, a weak Cat S inhibitor, were constructed and are represented in Figure 3 (see also SI Figures S6 and S7). Through charge−charge interaction, **6p** was predicted to utilize the electron-rich methyl ester group (CH_3OOC) at the P3 site to interact with the ammonium group of Lys64 in Cat S, whereas no such negatively charged group is available at the P3 site of **6t**. Compounds with other extended negatively charged substituents (NO_2, OCH_3, Cl, Br) at the P3 position also had better inhibitory activity than their counterparts lacking a charged substituent at P3. For example, the IC_{50} values of **6n** and **6o**, which contain an *N*-Boc moiety at the P3 site, are approximately 8-fold higher than those of **6p** and **6q**, respectively, which contain a NH_2 moiety at P3. Furthermore, formation of a unique hydrogen bond was predicted between the P3 amino group of **6p** and a carbonyl oxygen of Gly62 in Cat S, whereas **6t** has a rather large hydrophobic pyrene group at the P3 site which may limit its flexibility and its ability to inhibit Cat S. It should also be noted that the target compounds listed in Table 1 have a negative charge extending from the warhead (carbonyl group of the ethyl ester) available for interaction with the S1′ site (Arg141) of Cat S that subsequently stabilize the active site region. The docked models also suggest that an oxygen atom from the α-ketoamide of the inhibitor is near the sulfur atom of Cys25 of Cat S, which may allow formation of a reversible thiohemiketal bond.[11,24] This is supported by the observation that the IC_{50} values of the α-ketoamides are approximately 100- to 1900-fold lower than those of the corresponding α-hydroxyl-based structures. For example, the IC_{50} value of **6a** is 4.4 nM, whereas the corresponding α-hydroxyl compound **5** is greater than 10 μM. The double-reciprocal plot shown in SI Figure S8, in which Z-VVR-AMC (AMC, 7-amino-4-methyl coumarin) is used as a substrate, confirms that **6a** is a good competitive inhibitor of Cat S.

Conclusion

This report describes the design and synthesis of a series of noncytotoxic α-ketoamide compounds. These small molecules target and suppress the proteolytic activity of cathepsin S, a pivotal enzyme involved in the progression of cancer. A 96-well ELISA-based method for rapid synthesis and effective activity measurement was developed at the present study. The screening results revealed that some 30 compounds from 789 α-hydroxyl amides exert potent Cat S inhibition with IC_{50} lower than 10 nM. In particular, compounds **6i**, **6p**, and **6r** were found to not only decrease cell migration and invasiveness but also inhibit endothelial tube formation. Such promising in vitro characteristics suggest that the newly synthesized α-ketoamide Cat S inhibitors may give rise to alternative drug therapies to prevent or delay the spread of malignant tumor cells.

Experimental Section

Glycine, *N*-[(1,1-Dimethylethoxy)carbonyl]-cyclohexyl-L-alanyl-(2*S*)-3,4,5-trideoxy-3-[[(1,1-dimethylethoxy)carbonyl]amino]-4-benzyloxy-L-glycero-butanoyl, Ethyl Ester (3). Aldehyde **2** (1.0 g, 3.6 mmol), Boc-Cha-OH (0.981 g, 3.9 mmol), and ethyl isocyanoacetate (432 μL, 3.9 mmol) were dissolved in dry CH_2Cl_2 (20 mL), and the resulting solution was stirred for 48 h at room temperature (rt). The mixture was then concentrated and purified by silica gel chromatography (hexane/EtOAc = 2/1, R_f = 0.5) to give isomers **3** as a yellow syrup (1.8 g, 75%). FAB-HRMS (*m*/*z*) $[M + H]^+$ 624.3489. Calcd for $[C_{31}H_{50}N_3O_{10}]^+$: 624.3496.

Glycine, *N*-[(3*S*)-4-Benzyloxy-3-[[[1-[(4-morpholinylcarbonyl)-amino]cyclohexyl-L-alanyl]carbonyl]amino]-2-hydroxybutanoyl], Ethyl Ester (5a). Compound **3** (1.8 g, 2.7 mmol) was dissolved in 50% TFA (5 mL) in CH_2Cl_2 (10 mL), and the resulting solution was stirred for 30 min and then concentrated. The residue was redissolved in CH_2Cl_2 and neutralized with triethylamine. The solution was stirred for 10 min and then concentrated. The resulting mixture was dried under vacuum to give isomers **4**, which were used for next step without further purification. To a solution of compound **4** (0.4 g, 0.9 mmol), 4-morpholinecarbonyl chloride (0.1 mL, 1.0 mmol) in DMF (9 mL) was added Et_3N (0.4 mL, 2.7 mmol) and the resulting solution was stirred for overnight. The solvent was removed under reduced pressure, and the resulting mixture was diluted with EtOAc. The organic layer was washed with water, dried over $MgSO_4$, filtered, and concentrated. The mixture was purified by silica gel chromatography (EtOAc, R_f = 0.2) to give **5a** as a white syrup (415 mg, 82%). ESI-HRMS (*m*/*z*) $[M + H]^+$ 577.3228. Calcd for $[C_{29}H_{45}N_4O_8]^+$: 577.3237.

Glycine, *N*-[(3*S*)-4-Benzyloxy-3-[[[1-[(4-morpholinylcarbonyl)-amino]cyclohexyl-L-alanyl]carbon-yl]amino]-1,2-dioxobutyl], Ethyl Ester (6a). To a solution of compound **5a** (86 mg, 0.15 mmol) in CH_2Cl_2 (2 mL) was added Dess−Martin periodinane (0.19 g, 0.45 mmol), and the resulting solution was stirred for 2 h at rt. The mixture was quenched with $Na_2S_2O_3$ in saturated bicarbonate solution (2 mL). The residue was extracted with CH_2Cl_2. The organic layer was washed with H_2O, dried over $MgSO_4$, filtered,

and concentrated. The residue was purified by silica gel chromatography (EtOAc, $R_f = 0.4$) to give **6a** as a white syrup (76 mg, 89%; purity >99%). ^{1}H NMR ($CDCl_3$, 400 MHz, mixture of two diastereomers) δ 7.51−7.46 (m, 1H), 7.33−7.17 (m, 5H), 7.09 (d, J = 5.8 Hz, 1H), 5.46−5.42 (m, 1H), 5.12 (s, 1H), 4.52−4.45 (m, 1H), 4.42 and 4.44, 4.38 (s and d, J = 13.8 and 12.0 Hz, 2H), 4.20 and 4.19 (q, J = 7.2 and 7.2 Hz, 2H), 4.14 and 4.13 (dd, J = 9.8, 3.6 and 10.0, 3.8 Hz, 1H), 4.00−3.97 (m, 2H), 3.76 and 3.74 (t, J = 3.3 and 3.3 Hz, 1H), 3.63−3.60 (m, 4H), 3.35−3.28 (m, 4H), 1.74 (d, J = 12.6 Hz, 1H), 1.66−1.57 (m, 5H), 1.51−1.44 (m, 1H), 1.36−1.29 (m, 1H), 1.26 and 1.25 (t, J = 7.1 Hz, 3H), 1.22−1.04 (m, 3H), 0.96−0.78 (m, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz, mixture of two diastereomers) δ 193.0, 192.9, 173.5, 168.6, 159.2, 157.5, 157.3, 137.2, 128.3, 127.8, 127.6, 127.6, 73.3, 69.4, 69.3, 66.4, 61.8, 55.5, 55.4, 52.1, 51.9, 44.0, 43.9, 41.0, 40.3, 40.0, 34.1, 34.0, 33.5, 32.8, 32.7, 26.3, 26.1, 26.0, 14.1. ESI-HRMS (m/z) $[M + H]^+$ 575.3090. Calcd for $[C_{29}H_{43}N_4O_8]^+$: 575.3081.

Glycine, *N*-[(3*S*)-4-Benzyloxy-3-[[[((2*S*)-2-[(3-amino-4-chlorobenzoyl)amino]cyclohexyl-L-alanyl]carbonyl]amino]-1,2-dioxobutyl], Ethyl Ester (6q). To a solution of compound **6o** in CH_2Cl_2 (0.5 mL) was added TFA (0.5 mL) at 0 °C. The resulting solution was stirred for 30 min and then concentrated. The residue was purified by silica gel chromatography (hexane/EtOAc = 1/1, R_f = 0.3) to give **6p** as a white syrup (0.022 g, 78%; purity >99%). ^{1}H NMR ($CDCl_3$, 400 MHz, mixture of two diastereomers) δ 7.36 (t, J= 5.1 Hz, 1H), 7.28−7.21 (m, 5H), 7.19−7.17 (m, 2H), 7.10 and 6.95 (d, J = 7.2 and 7.6 Hz, 1H), 6.99 (dd, J = 8.2, 2.0 Hz, 1H), 6.64 (d, J = 8.1 Hz, 1H), 5.47−5.44 (m, 1H), 4.77−4.70 (m, 1H), 4.43 and 4.43, 4.39 (s and d, J = 11.8 and 12.0 Hz, 2H), 4.22 (q, J = 7.2 Hz, 2H), 4.16 (dd, J = 9.8, 3.5 Hz, 1H), 4.01 and 3.98 (dd and d, J = 5.4, 3.2, and 5.6 Hz, 2H), 3.78 and 3.76 (dd, J = 9.9, 3.3 Hz, 1H), 1.81−1.56 (m, 7H), 1.35 (s, 1H), 1.28 (t, J = 7.2 Hz, 3H), 1.25−1.14 (m, 3H), 1.01−0.86 (m, 2H). FAB-HRMS (m/z) $[M + H]^+$ 615.2592. Calcd for $[C_{31}H_{40}N_4O_7Cl]^+$: 615.2586.

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Supporting Information Available: Experimental details for chemistry, purity, retention time, and characterization of target compounds; enzyme inhibition and cell-based bioassays. This material is available free of charge via the Internet at http://pubs.acs.org.

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