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Novel matrix metalloproteinase inhibitors: Generation of lead compounds by the in silico fragment-based approach

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Abstract—Generation of structurally new matrix metalloproteinase inhibitors was successfully carried out using an in silico technique. In order to identify the small fragment interacting with residues in the S1′ pocket of MMP-1 through hydrogen bonds, we performed in silico screening using the LUDI program. As a result, acetyl-L-alanyl-(*N*-methyl)amide (Ac-L-Ala-NHMe) was selected to link with another fragment, hydroxamic acid that interacted with catalytic zinc. By this approach, the L-glutamic acid derivative **2b** was discovered to be a new type of matrix metalloproteinase inhibitor. Further transformation to reduce its peptidic nature and improve activity yielded nonpeptidic lead compounds as inhibitors of MMP-1, -2, -3, and -9.

1. Introduction

Matrix metalloproteinases (MMPs), a family of zinc-containing endopeptidases, have been shown to play a central role in various physiological and pathophysiological events, such as embryonic development, blastocyte implantation, nerve growth, ovulation, morphogenesis, angiogenesis, tissue resorption, and tissue remodeling. In addition, the overexpression or dysregulation of MMPs is associated with several disease processes, such as tumor metastasis, arthritis, artherosclerosis, aneurysm, breakdown of the blood–brain barrier, periodontal disease, skin ulcers, corneal ulcers, gastric ulcers, and liver fibrosis. In patients with these diseases, MMPs are considered to be a potential drug target. In fact, a number of MMP inhibitors (MMPIs) have been developed, several of which have shown some efficacy in clinical trials.[1]

Inhibition of MMPs is related to the coordination between the inhibitor molecule (generally as an anion) and the catalytic metal ion, with or without substitution of the metal-bound water molecule. MMPIs should have a zinc-binding moiety attached to a substructure that interacts with the substrate recognition sites of the enzyme. The strongest known class of MMPIs is the hydroxamates, which have been shown to bind bidentately to the catalytic Zn(II) of the enzyme, causing it to adopt a distorted trigonal bipyramidal geometry.[2] The hydroxamate anion forms a short and strong hydrogen bond with the carboxylate moiety of Glu 219, which is oriented toward the unprimed binding regions, whereas the NH hydroxamate participates in a hydrogen bond with the carbonyl oxygen of Ala 182. Thus, several strong interactions occur at the zinc site without any significant unfavorable contacts.

To discover the substructure interacting with the substrate recognition sites of MMPs, NMR-based screening has proven to be a valuable approach. For this approach, individual small compounds are mixed with a ^{15}N-labeled protein to evaluate their binding affinity. By this method, 4-cyanobiphenyl was found to show weak binding to the S1′ pocket in the case of stromelysin (MMP-3), and then was connected with hydroxamic acid through an appropriate linker to successfully produce potent inhibitors of MMP-3. Although this NMR-based fragment approach is a powerful method of discovering lead compounds, a significant amount of isotope-labeled protein is required for screening.[3]

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In silico virtual screening of small molecules based on the three-dimensional structure of the target protein is an attractive way to identify candidates that may interact with a specific site. As structural information about the target protein is established in more detail, this in silico approach becomes increasingly reliable. In the case of MMPs, detailed three-dimensional structural information about the binding sites of MMPIs is available from X-ray crystallography, NMR experiments, and structure–activity relationship (SAR) data. This situation prompted us to explore virtual screening with the de novo program, LUDI,[4] in order to identify new substructures that might interact with amino acids around the S1′ pocket of MMPs. Here, we report the results of our in silico fragment-based approach and the preliminary SAR data for the identified lead compounds.

2. Chemistry

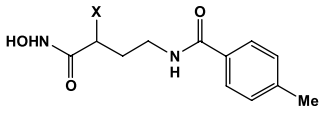
Synthesis of the test compounds listed in Tables 1–4 is described in Schemes 1–8. Synthesis of compounds **2a**,**b**, and **5** is outlined in Scheme 1. Condensation of the optically pure glutamic acid derivatives **19a**,**b** with methylamine afforded **20a**,**b**, respectively. Catalytic hydrogenation of **20a** provided **21a**, while acidic deprotection of **20b** gave **21b**. Acidic deprotection of **21a** resulted in **2a**, while catalytic hydrogenation of **21b** produced **22**. The carboxylic acids **2a** and **22** were converted to hydroxamic acids (**2b** and **5**) by the condensation with *O*-benzylhydroxyamine followed by catalytic hydrogenation.

Table 1. Activity profiles of newly designed chemical leads (Chart 1)

Compound	IC_{50} (μM)			
	MMP-1	MMP-2	MMP-9	MMP-3
2a	>100	>100	NT[b]	>100
2b	19	2.4	38	39%[a]
3	>100	>100	>100	>100
4	59%[a]	18	40%[a]	NT
5	>100	>100	NT	>100
6	>100	>100	NT	>100

[a] Inhibition percentage at 100 μM.
[b] Not tested.

Table 3. Effect of the C2 substitution of 4-aminobutyric acid analogs on the activity profiles



Compound	X	IC_{50} (μM)			
		MMP-1	MMP-2	MMP-9	MMP-3
4	H	59%[a]	18	40%[a]	NT
10	CH_2OH	6.4	4.0	NT	NT
11	(+)-CH_2OH	88%[a]	2.2	11	54
12	(−)-CH_2OH	69%[a]	12	38	>100
13	CH_2OBzl	0.53	0.18	1.2	5.7

[a] Inhibition percentage at 100 μM.

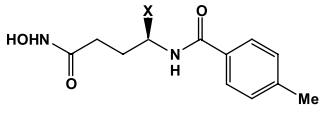
Compound **3** was prepared as outlined in Scheme 2. *N*-Acylation of **25**, which was prepared from **24**, with a glutaric anhydride followed by formation of the *N*-methylamide by the conventional procedures afforded **26b**. Without *N*-protection in **26a**, an exclusive intramolecular cyclization took place in the following *N*-methylamide formation reaction. Deprotection of **26b** with a catalytic hydrogenation resulted in **3**.

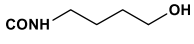
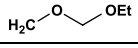
Compound **4** was synthesized as outlined in Scheme 3. *N*-Acylation of 4-aminobutyric acid with 4-methylbenzoylchloride in the presence of sodium hydroxide gave **28**, which was converted to the corresponding hydroxamic acid **4** by the usual method.

Compound **6** was also synthesized from **19b** as outlined in Scheme 4. Condensation of **19b** with 4-methyl aniline afforded **30**, catalytic hydrogenation of which gave **31**. Condensation of **31** with *O*-benzylhydroxyamine produced **32**. Acidic deprotection of **32** resulted in **33**, which was converted to **34** according to the usual *N*-acetylation. Deprotection of **34** by catalytic hydrogenation produced the hydroxamate **6**.

Synthesis of **7**–**8** is described in Scheme 5. *N*-Acylation of a protected glutamic acid **35** with 4-methylbenzoyl chloride afforded **36**, deprotection of which by catalytic hydrogenation provided the carboxylic acid **37**. Condensation of **37** with **25** gave **38**. Acidic deprotection of **38**

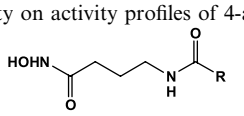
Table 2. Effect of the chemical modification of C1 carboxylic acid moiety of L-glutamic acid analog on the activity profiles

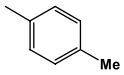
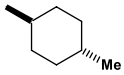
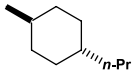
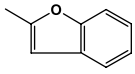
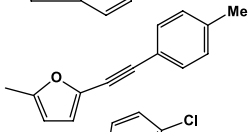
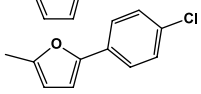


Compound	X	IC_{50} (μM)			
		MMP-1	MMP-2	MMP-9	MMP-3
2b	CONHMe	19	2.4	38	39%[a]
7	COOH	NT	14	NT	>100
8		23	3.4	NT	41%[a]
9		4.3	>1	>1	84%[a]

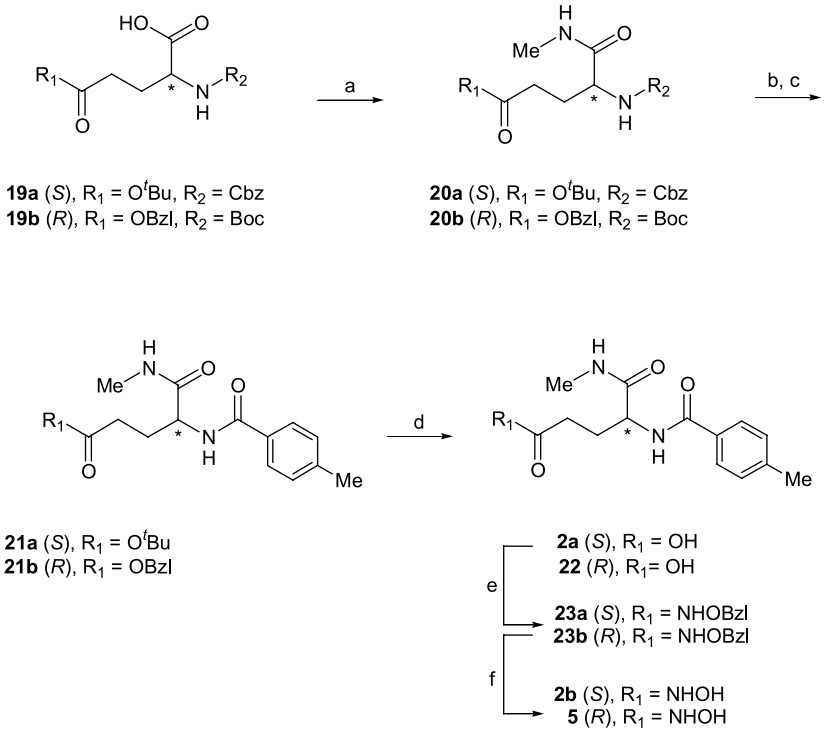
[a] Inhibition percentage at 100 μM.

Table 4. Effect of chemical modification of the *N*-acyl moiety on activity profiles of 4-aminobutyric acid analogs



Compound	R	IC_{50} (μM)			
		MMP-1	MMP-2	MMP-9	MMP-3
4		59%[a]	18	40%[a]	NT
14		9.6	47%[a]	NT	>100
15		67%[a]	6.8	NT	65%[a]
16		>100	45%[a]	NT	>100
17		>100	2.8	NT	13
18		>100	3.5	NT	1.0

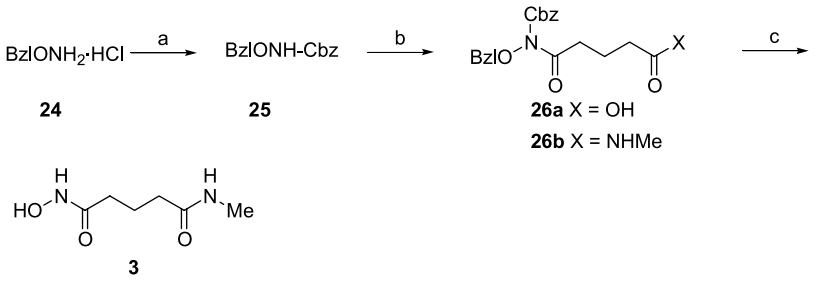
[a] Inhibition percentage at 100 μM.



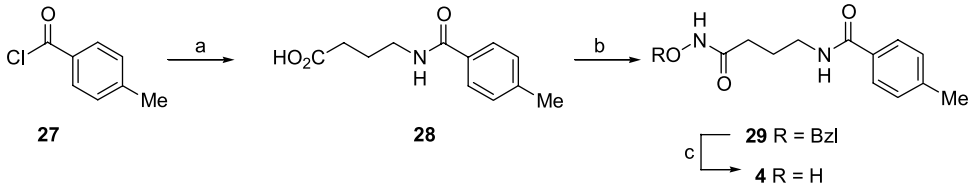
Scheme 1. Synthesis of **2a**,**b**, and **5**. Reagents: (a) (i) $ClCO_2{}^iBu$, NMM, THF; (ii) NH_2Me; (b) H_2, Pd–C, MeOH or 4 N HCl/dioxane; (c) 4-methylbenzoyl chloride, pyridine or 4-methylbenzoyl chloride, Et_3N, CH_2Cl_2; (d) TFA–H_2O or H_2, Pd–C, EtOH; (e) HCl·NH_2OBzl, EDC, HOBt, Et_3N, DMF; (f) H_2, Pd–C, MeOH.

resulted in **39**, catalytic hydrogenation of which led to **7**. Condensation of **39** with 4-amino-1-butanol afforded **40**, after which deprotection by the usual method produced **8**.

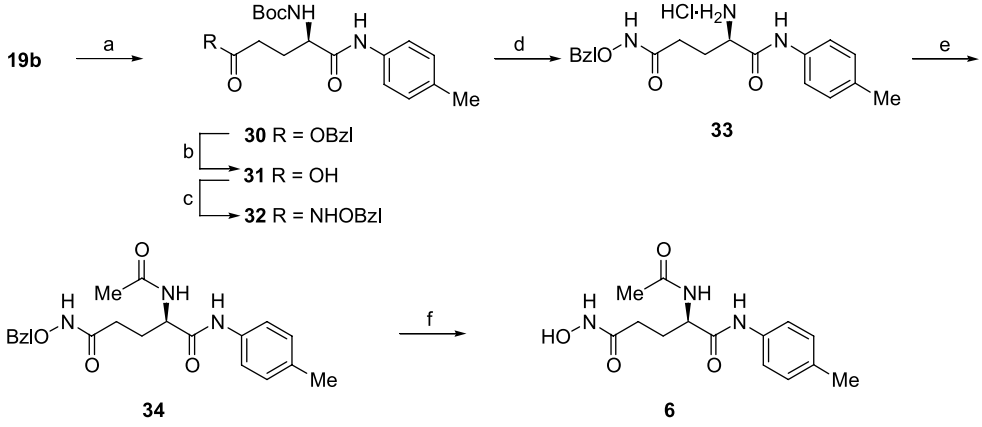
Synthesis of **9** is outlined in Scheme 6. A commercially available protected glutamic acid **41** was converted to **45** by the following sequence of reactions. Formation of an activated ester of **41**, followed by sodium borohy-



Scheme 2. Synthesis of **3**. Reagents: (a) Cbz–Cl, $^{i}Pr_2NEt$, CH_2Cl_2; (b) (i) glutaric anhydride, DMAP, CH_3CN; (ii) H_2NMe, EDC, HOBt, $^{i}Pr_2NEt$, DMF; (c) H_2, Pd–C, MeOH.



Scheme 3. Synthesis of **4**. Reagents: (a) 4-aminobutyric acid, NaOH aq, THF; (b) HCl·NH_2OBzl, EDC, HOBt, $^{i}Pr_2NEt$, DMF; (c) H_2, Pd–C, MeOH.



Scheme 4. Synthesis of **6**. Reagents: (a) 4-methylaniline, EDC, HOBt, Et_3N, DMF; (b) H_2, Pd–C, EtOAc; (c) HCl·NH_2OBzl, EDC, HOBt, Et_3N, DMF; (d) 4 N HCl/dioxane; (e) Ac_2O, $^{i}Pr_2NEt$; (f) H_2, Pd–C, MeOH.

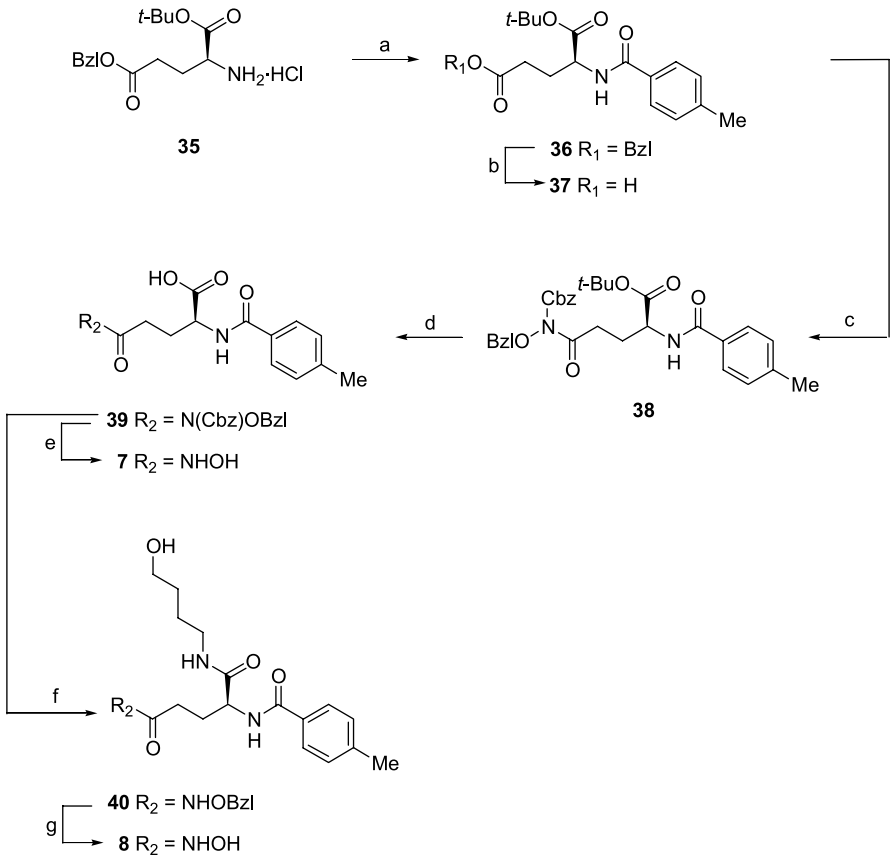
dride reduction, gave **42**, after which *O*-alkylation with ethoxyethylchloride provided **43**. Catalytic hydrogenation of **43**, followed by *N*-acylation with 4-methylbenzoyl chloride, afforded **44**, after which alkaline hydrolysis resulted in **45**. Condensation of **45** with *O*-(2-methoxypropane-2-yl)hydroxylamine followed by acidic deprotection produced **9**.

Synthesis of **10**–**13** is described in Scheme 7. *C*2-Alkylation of a 4-amino-*N*-benzoyl-butyric acid **28** with benzyloxymethylchloride afforded **46**, condensation of which with *O*-benzyl hydroxylamine provided **47**. Partial hydrogenolysis of **47** gave **13**, which was converted to an alcohol **10** by treatment with boron tribromide. After optical resolution of **47** using a chiral HPLC coloumn, the resulting two enantiomers were deprotected by catalytic hydrogenation to produce **11** and **12**, respectively.

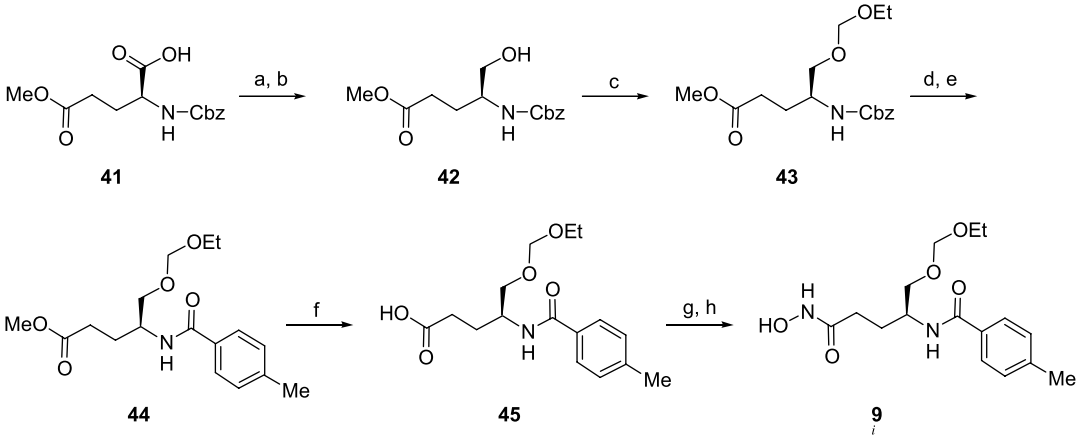
Compounds **14**–**18** were synthesized as described in Scheme 7. Condensation of carboxylic acids **48a**–**e** with 4-aminobutyric acid ethyl ester afforded **49a**–**e**, alkaline hydrolysis of which gave **50a**–**e**, respectively. Condensation of **50a**–**e** with *O*-(2-methoxypropane-2-yl)hydroxylamine, followed by acidic deprotection, produced **14**–**18**, respectively.

3. In silico generation of lead compounds

In order to design new MMP inhibitors, we selected the strategy of connecting two fragments by an appropriate linker. The first fragment was intended to interact with the catalytic Zn(II) ion. Based on information about known MMPIs, we selected formic acid and *N*-formyl hydroxylamine as candidate fragments. The second fragment was expected to form hydrogen bonds around



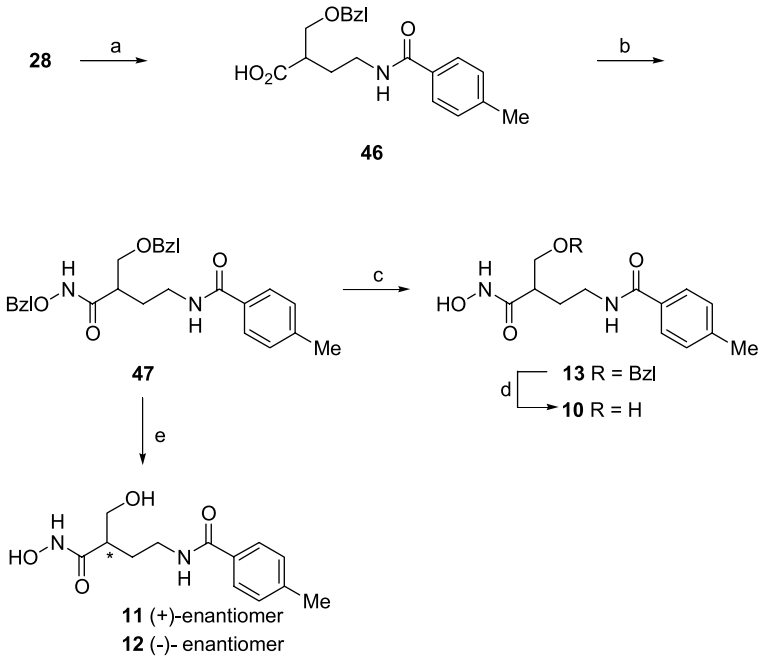
Scheme 5. Synthesis of **7** and **8**. Reagents: (a) 4-methylbenzoyl chloride, $^{i}Pr_2NEt$, $CHCl_3$; (b) H_2, Pd–C, EtOAc; (c) **25**, EDC, DMAP, DMF; (d) TFA (e) H_2, Pd–C, MeOH; (f) 4-amino-1-butanol, EDC, HOBt, Et_3N, DMF; (g) H_2, Pd–C, MeOH.



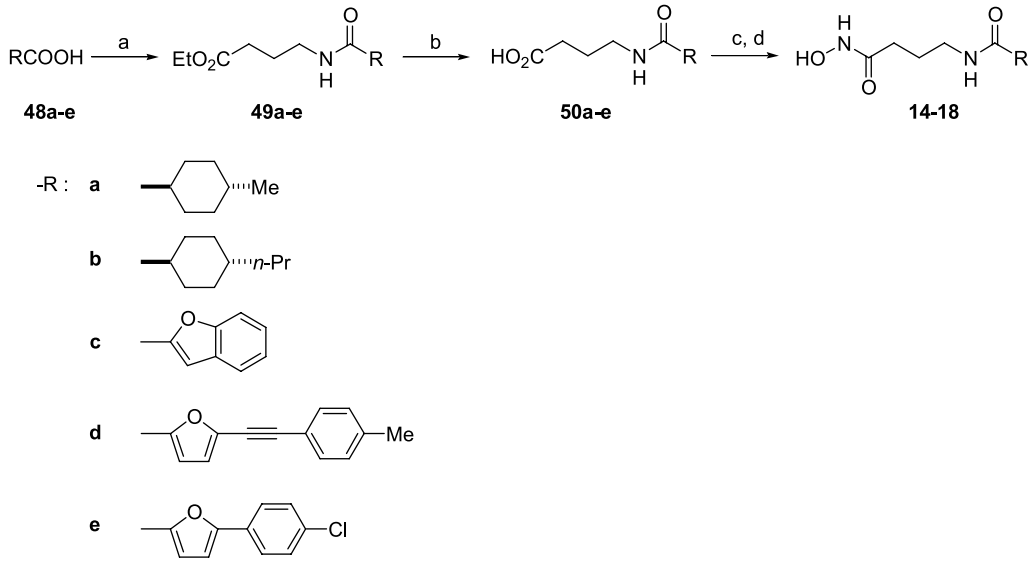
Scheme 6. Synthesis of **9**. Reagents: (a) HONSu, DCC, THF; (b) $NaBH_4$, THF–H_2O; (c) $EtOCH_2Cl$, $^{i}Pr_2NEt$, CH_2Cl_2; (d) H_2, Pd–C, MeOH; (e) 4-methylbenzoyl chloride, Et_3N, CH_2Cl_2; (f) NaOH aq, MeOH; (g) *O*-(2-methoxypropane-2-yl)hydroxylamine, EDC, HOBt, DMF; (h) HCl, MeOH.

the S1′ pocket and to be involved in the van der Waals interaction with the pocket. LUDI was used as a de novo program to detect fragments with the possibility of showing a second interaction with collagenase (MMP-1).[5]

From the X-ray crystallography and the well-established SAR data on inhibition of MMPs by numerous inhibitors, detailed three-dimensional structural information is available about the binding sites of MMPIs. Most MMPIs bind to the same enzyme pocket and occupy common subregions. Around the S1′ pocket, they all show a common interaction with Pro238 and Leu181. Each MMPI donates a hydrogen bond to a proline residue (Pro238) and accepts a hydrogen bond from a leucine residue (Leu181). We reasoned that a novel inhibitor should have the ability to form these two key hydrogen bonds. It should also contain a lipophilic S1′



Scheme 7. Synthesis of **10**–**13**. Reagents: (a) LDA, $BzlOCH_2Cl$, HMPA, THF; (b) $HCl{\cdot}NH_2OBzl$, EDC, HOBt, $^iPr_2NEt$, DMF; (c) H_2, Pd–C, MeOH; (d) BBr_3, CH_2Cl_2; (e) HPLC separation then, H_2, Pd–C, MeOH.



Scheme 8. Synthesis of **14**–**18**. Reagents: (a) 4-aminobutyric acid ethyl ester hydrochloride, EDC, HOBt, Et_3N, DMF; (b) NaOH aq, MeOH; (c) *O*-(2-methoxypropane-2-yl)hydroxylamine, EDC, HOBt, DMF; (d) HCl, MeOH.

pocket to satisfy the steric and lipophilic requirements of the enzyme pocket.

A search of the standard LUDI library was carried out to identify candidate second fragments. This search resulted in a list of 83 small molecules. Although *N*-acetyl-L-alanine *N*-methylamide (Ac-L-Ala-NHMe) had a relatively low LUDI score, we tested it because of the ease of synthesis and the orientation of the α-methyl group. A docking study of Ac-L-Ala-NHMe with MMP-1[5] suggested the formation of key hydrogen bonds with Pro238 and Leu181 (Fig. 1). As the methyl moiety of Ala was suggested to be directed towards the zinc site, we decided to connect this methyl group with the first fragment using a simple linker (methylene group). It was also thought that the methyl moiety of *N*-acetyl should be located in the lipophilic S1′ pocket. However, this methyl group was too small to fill the cavity, so we replaced it with a 4-methylphenyl group. Based on the above-mentioned analysis, we designed the glutamic acid derivatives **2a**,**b** and performed a docking study. As shown in Figure 2, compound **2b** seemed to fit the active site of MMP-1.

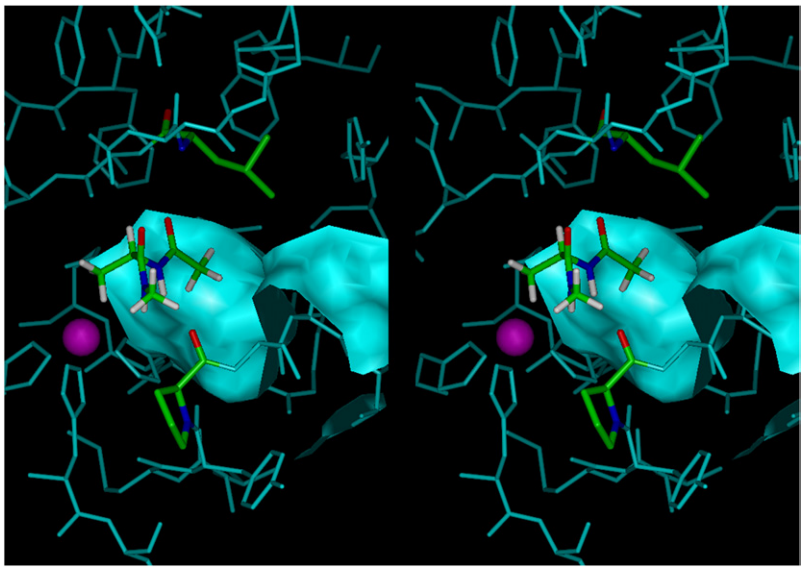


Figure 1. Docking study of Ac-L-Ala-NHMe **1** with MMP-1.

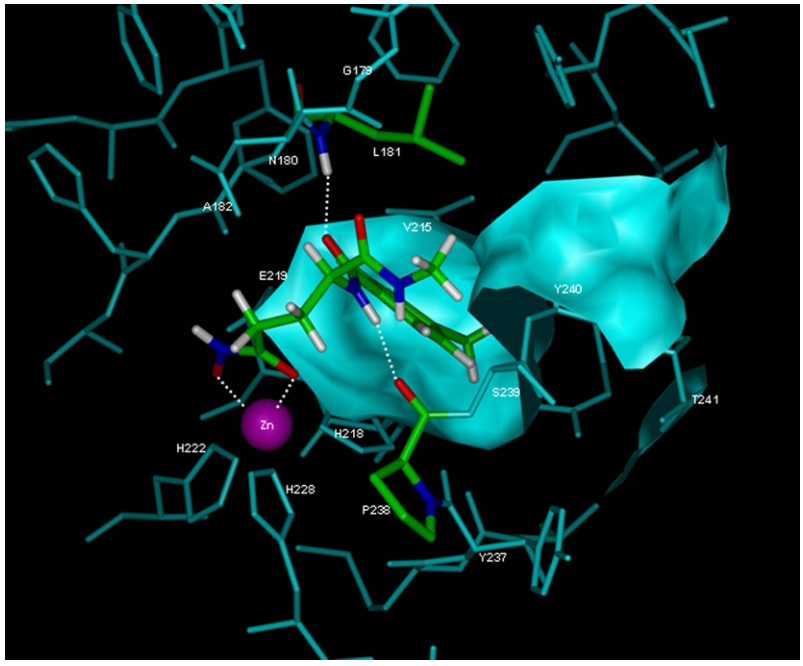


Figure 2. Docking study of **2b** with MMP-1.

Based on the promising outcome of in silico simulation, compounds **2a**,**b** were synthesized, and their inhibition of MMP-1, -2, -9, and -3 was measured in vitro. The carboxylic acid **2a** did not show any significant inhibitory activity at 100 μM, but the hydroxamic acid **2b** did demonstrated moderate to weak inhibitory activity (Table 1).

4. Structure–activity relationships

As shown in Table 1, the effect of chemical modification of the two amide moieties of **2b** on the activity profile was investigated in order to confirm the results of in silico modeling. The glutaric acid derivative **3** showed complete loss of inhibitory activity, but the 4-aminobutyric acid derivative **4** still showed weak inhibition of MMP-1, MMP-2, and MMP-9. This result indicated that the benzamide moiety is more important for interaction with the target enzyme than the formamide moiety. Conversion of the 2*S*-configuration of **2b** to a 2*R*-configuration gave **5**, with loss of inhibitory activity against MMP-1, MMP-2, and MMP-3 at 100 μM. This indicated that the 2*S*-configuration of **2b** plays a role in supporting the interaction with the target enzyme, while the 2*R*-configuration of **5** seems to disturb interaction with the enzyme. In order to confirm the importance of hydrogen bonds, a retro-inverse analogue of **2b** was synthesized and evaluated for

inhibition of MMP-1, -2, and -3. This analogue **6** did not inhibit the MMPs at 100 μM at all. These results supported the concept that compound **2b** showed its activity through hydrogen bond interactions at the benzamide moiety (Chart 1).

Based on the above information, further optimization was conducted without chemical modification of the hydroxamate moiety. The docking study of **2b** with MMP-1 indicated that the *N*-methylformamide moiety might not interact with the enzyme and could be located in the aqueous environment. Accordingly, its replacement of this part with a hydrophilic substituent was investigated. As shown in Table 2, conversion to the carboxylic acid moiety gave **7**, which showed reduced MMP-2 inhibitory activity. Replacement of the methyl group with a 4-hydroxybutyl moiety resulted in **8**, with retention of the inhibitory activity against MMP-1 and MMP-2. In order to reduce the peptidic nature and maintain hydrophilicity, the *N*-methylformamide moiety was replaced with a 1-ethoxymethoxymethyl moiety. The resulting compound **9** showed an increase of inhibitory activity against MMP-1 and MMP-3.

Hydroxamate-containing compounds are known to be susceptible to hydrolysis to form the corresponding carboxylic acids in vivo. One promising approach to prevent such hydrolysis is the introduction of a substituent near the hydroxamate moiety, so the effect on the activity profile of incorporation of a hydroxymethyl group at the α-position of 4-aminobutyric acid analog **4** was investigated. Fortunately, introduction of a hydroxymethyl moiety at the α-position of **4** gave **10**, which showed an increase of inhibitory activity against MMP-1 and MMP-2. (+)-Isomer **11** showed more potent inhibitory activity against all of the tested isoforms than (−)-isomer **12**. Introduction of a benzyloxymethyl moiety at the α-position of **4** afforded **13**, which showed a significant increase of inhibitory activity against all the MMP isoforms tested. As a result, the carbon next to hydroxamate was also found to be another site for further chemical modification as illustrated by **13**.

A docking study suggested that the benzamide moiety of **2b** might be located in the S1′ cavity of the enzyme, as shown in Figure 2. Although all MMPs have a similar environment around the active site, there are slight differences of the environment in the S1′ pocket, for example, the S1′ cavities of MMP-2, -3, and -9 are known to be deeper and narrower than that of MMP-1.[1] To ascertain whether the benzamide moiety of **2b** was located in the S1′ cavity, **14**–**18** were synthesized and their inhibitory activity was evaluated. Replacement of the 4-methylbenzoyl moiety of **4** with a *trans*-4-methylcyclohexanoyl moiety gave **14**, which showed increased activity against MMP-1 and reduced activity against MMP-2. Conversion of the methyl group of **14** to an *n*-propyl group afforded **15**, which showed a decrease of activity against MMP-1 and an increase of activity against MMP-2. Replacement of the 4-methylbenzoyl moiety of **4** with a benzofuran-2-yl moiety provided **16**, with loss of inhibitory activity against MMP-1, -2, and -3, conversion to the 5-(4-methylphenylethynyl)furan-2-yl moiety or the 5-(4-chlorophenyl)furan-2-yl moiety resulted in **17** or **18** and both showed a significant increase of inhibitory activity against MMP-2 and MMP-3. A similar trend was observed in the case of substrate-based inhibitors.[1a] While the selective MMP-1 inhibitors had short and bulky substituents such as cyclohexyl or isobutyl groups at the P1′ site, the selective MMP-2 or MMP-3 inhibitors had long substituents like biphenyl or 4-phenylbutyl groups at that site. These results strongly suggested that the benzamide moiety was located in the S1′ cavity.

In summary, we succeeded in the generation of structurally new lead compounds that acted as MMPIs using the in silico fragment approach. As shown in Figure 3, **2b** was designed by replacing the *N*-acetyl residue of compound **1** with a 4-methylbenzoyl residue, followed by connection of the methyl moiety of the Ala unit of compound **1** with an *N*-formylhydroxylamine by a simple linker, methylene. Compounds **2b**, **8**, and **11** inhibited MMP-2 selectively, while **9** and **14** inhibited MMP-1 selectively relative to the other MMP isoforms. Compound **13** inhibited all of the MMP isoforms although its inhibitory activity against MMP-1 and MMP-2 was

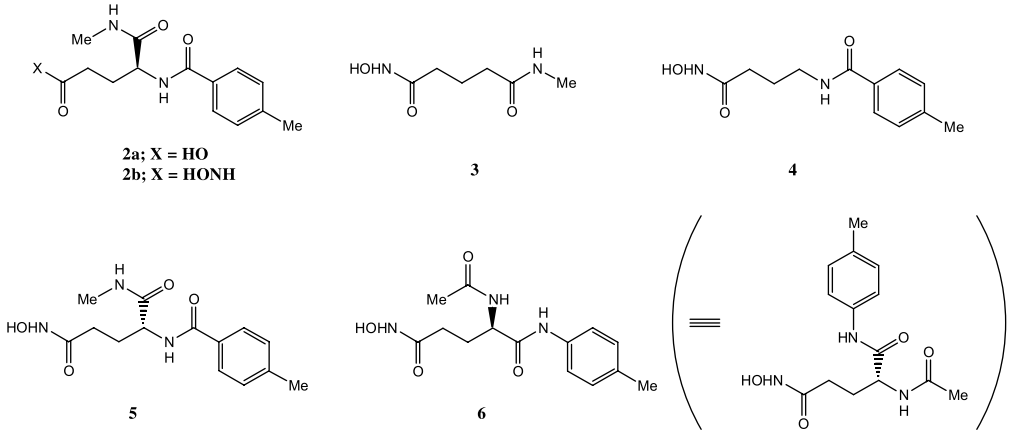


Chart 1. New chemical leads for MMPIs.

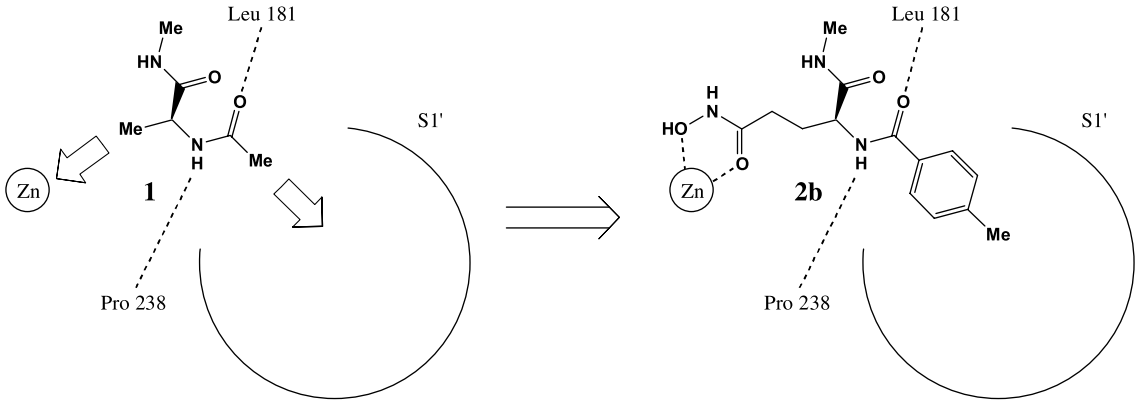


Figure 3. Molecular design of a L-glutamic acid analog **2b**.

stronger than that against MMP-9 and MMP-3. These new L-glutamic acid or 4-aminobutyric acid-based structures should undergo diverse chemical modifications to identify MMP isoform-selective inhibitors because of cost-effectiveness and good functionality. Further optimization of these compounds will be reported in the near future.

5. Experimental

Analytical samples were homogeneous as confirmed by thin-layer chromatography (TLC), and yielded spectroscopic data consistent with the assigned structures. All ^{1}H NMR spectra were obtained with a Varian Gemini-200 or MERCURY-300 spectrometer. The chemical shift values are reported in ppm (δ) and coupling constants (J) in hertz (Hz). Fast atom bombardment (FAB) and electron ionization (EI) mass spectra were obtained with a JEOL JMS-DX303HF or JMS-700 spectrometer. Atmospheric pressure chemical ionization (APCI) mass spectra were determined by a Hitachi M-1200H spectrometer. Matrix-assisted laser desorption ionization (MALDI) mass spectra were obtained on a PerSeptive Biosystems Voyager™ Elite spectrometer. IR spectra were measured using a Perkin–Elmer FTIR 1760X or JASCO FTIR-430 spectrometer. Column chromatography was carried out using silica gel [Merck silica gel 60 (0.063–0.200 mm), Wako Gel C200, Fuji Silysia FL60D, or Fuji Silysia BW-235]. TLC was also performed on silica gel (Merck TLC plate, silica gel 60 F254). The following abbreviations for solvents and reagents are used: THF, tetrahydrofuran; EtOAc, ethylacetate; MeOH, methanol; EtOH, ethanol; DMF, *N*,*N*-dimethylformamide; CH_2Cl_2, dichloromethane; $CHCl_3$, chloroform; EDC·HCl, 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride; HOBt, 1-hydroxybenzotriazole; HMPA, hexamethylphosphoramide; TFA, trifluoroacetic acid; DMSO, dimethylsulfoxide; AcOH, acetic acid; Et_2O, diethylether; BuLi, butyllithium; FITC, fluorescein isothiocyanate; MOCAc, 7-methoxycoumarin-4-acetyl; Dpa, *N*-3-(2,4-dinitrophenyl)-L-2,3-diaminopropionyl; Dnp, 2,4-dinitrophenyl.

5.1. *tert*-Butyl N^2-[(benzyloxy)carbonyl]-N^1-methyl-L-glutaminate (20a)

To a stirred solution of 5-*tert*-butyl *N*-carbobenzoxy-L-glutamate (**19a**) (1.00 g, 2.97 mmol) in THF (6 mL) were added *N*-methylmorpholine (0.40 mL, 3.6 mmol) and isobutyl chloroformate (0.50 mL, 3.6 mmol) at −20 °C. After stirring at −20 °C for 15 min, excess amount of aqueous methylamine solution (40%) was added to the mixture, and then it was warmed to room temperature. The reaction mixture was diluted with EtOAc and washed with 1 N HCl, saturated $NaHCO_3$, and brine. The organic layer was dried over $MgSO_4$. Removal of the volatiles in vacuo provided a solid, which was purified by silica gel chromatography with MeOH–$CHCl_3$ (3:97) to give the title compound **20a** in quantitative yield as a white powder: TLC R_f = 0.73 ($CHCl_3$–MeOH, 9:1); MS (APCI, pos. 20 V) *m*/*z* 351 $(M+H)^+$; ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.38–7.31 (m, 5H), 6.24 (br s, 1H), 5.65 (m, 1H), 5.10 (s, 2H), 4.20–4.12 (m, 1H), 2.81 (d, J = 5.0 Hz, 3H), 2.49–2.24 (m, 2H), 2.17–1.85 (m, 2H), 1.44 (s, 9H).

5.2. *tert*-Butyl N^1-methyl-N^2-(4-methylbenzoyl)-L-glutaminate (21a)

Catalytic hydrogenation of **20a** (1.18 g, 3.37 mmol) in MeOH (12 mL) was conducted at room temperature for 21 h in the presence of 5% palladium on carbon (50 mg) at atmospheric pressure. Removal of the catalyst by filtration followed by evaporation of the solvent afforded the amine in 98% yield as a white amorphous powder: TLC R_f = 0.29 ($CHCl_3$–MeOH, 9:1). To a stirred solution of the above amine (500 mg, 2.31 mmol) in pyridine (3 mL) was added 4-methylbenzoyl chloride (0.30 mL, 2.8 mmol) at 0 °C. After stirring at room temperature overnight, the reaction mixture was diluted with EtOAc. The resulting solution was washed with 1 N HCl, saturated $NaHCO_3$, and brine, and dried over $MgSO_4$. Removal of the volatiles in vacuo provided an oily residue, which was purified by silica gel chromatography with MeOH–$CHCl_3$ (3:97) to give **21a** (499 mg, 65% yield) as a white powder: TLC R_f = 0.46 ($CHCl_3$–MeOH, 9:1); MS (APCI, pos. 20 V) 355 $(M+H)^+$. ^{1}H NMR

(200 MHz, $CDCl_3$): δ 7.74 (d, J = 8.0 Hz, 2H), 7.37 (d, J = 6.6 Hz, 1H), 7.24 (d, J = 8.0 Hz, 2H), 6.61–6.55 (m, 1H), 4.59 (q, J = 6.6 Hz, 1H), 2.84 (d, J = 4.9 Hz, 3H), 2.65–2.08 (m, 4H), 2.40 (s, 3H), 1.43 (s, 9H).

5.3. Benzyl N^2-(*tert*-butoxycarbonyl)-N^1-methyl-D-glutaminate (20b) and benzyl N^1-methyl-N^2-(4-methylbenzoyl)-D-glutaminate (21b)

The amide **20b** was obtained as a white powder in 80% yield from 5-benzyl *N*-(*tert*-butoxycarbonyl)-D-glutamate (**19b**) according to the analogous procedures as described for the preparation of **20a**. Compound **20b**: TLC R_f = 0.20 (EtOAc–*n*-hexane, 1:1). A solution of **20b** (3.34 g, 10.0 mmol) in 4 N HCl/dioxane (50 mL) was stirred at room temperature for 1 h. The reaction mixture was evaporated to give the amine hydrochloride quantitatively as a yellow oil. To a stirred solution of the above amine hydrochloride (10.0 mmol) in CH_2Cl_2 (50 mL) were added triethylamine (3.4 mL, 24 mmol) and 4-methylbenzoyl chloride (1.39 mL, 10.5 mmol) at 0 °C. After stirring at room temperature overnight, the solution was washed with 1 N HCl, saturated $NaHCO_3$, and brine, and dried over $MgSO_4$. Removal of the volatiles in vacuo provided **21b** (2.39 g, 65% yield) as a white powder: TLC R_f = 0.50 ($CHCl_3$–MeOH, 30:1); MS (EI, pos.) m/z 368 (M^+); ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.90–7.70 (m, 2H), 7.40–7.10 (m, 8H), 6.60–6.30 (br, 1H), 5.12 (s, 2H), 4.80–4.50 (m, 2H), 2.81 (d, J = 4.8 Hz, 3H), 2.40 (s, 3H), 2.80–2.10 (m, 3H).

5.4. N^1-Methyl-N^2-(4-methylbenzoyl)-L-α-glutamine (2a)

A solution of **21a** (499 mg, 1.49 mmol) in a mixed solvent of TFA–H_2O (3 mL:0.3 mL) was stirred at room temperature for 1 h. The reaction mixture was evaporated to give **2a** (416 mg, 100% yield) as a white powder: TLC R_f = 0.40 ($CHCl_3$–MeOH, 9:1); MS (FAB, pos.) m/z 279 $(M+H)^+$; IR (KBr) 3343, 2944, 1647, 1527, 1503, 1454, 1415, 1290, 1210, 946, 897, 834, 761, 653, 566, 534, 484 cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6): δ 12.13 (br s, 1H), 8.33 (d, J = 7.8 Hz, 2H), 7.86–7.78 (m, 3H), 7.26 (d, J = 8.1 Hz, 2H), 4.40–4.25 (m, 1H), 2.59 (d, J = 4.8 Hz, 3H), 2.36 (s, 3H), 2.30–2.23 (m, 2H), 2.05–1.82 (m, 2H); optical rotation $[\alpha]_D^{30}$ +5.65° (c 0.54, MeOH); HRMS (FAB) calcd for $C_{14}H_{19}N_2O_4$: 279.1345. Found: 279.1339.

5.5. N^1-Methyl-N^2-(4-methylbenzoyl)-D-α-glutamine (22)

Catalytic hydrogenation of **21b** (2.38 g, 6.46 mmol) in EtOH (65 mL) was conducted at room temperature for 1 h in the presence of 10% palladium on carbon (344 mg) at atmospheric pressure. Removal of the catalyst by filtration followed by evaporation of the solvent afforded **22** (1.63 g, 91% yield) as a white powder: TLC R_f = 0.20 ($CHCl_3$–MeOH, 5:1); MS (FAB, pos.) 279 $(M+H)^+$; IR (KBr) 3339, 3260, 1735, 1698, 1651, 1531, 1305, 1416, 1306, 1210, 947, 870, 835, 761, 692, 534 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$ + CD_3OD): δ 7.73–7.60 (m, 2H), 7.22–7.14 (m, 2H), 4.66–4.52 (m, 1H), 2.76 (s, 3H), 2.56–2.30 (m, 2H), 2.34 (s, 3H), 2.22–1.84 (m, 2H).

5.6. N^5-(Benzyloxy)-N^1-methyl-N^2-(4-methylbenzoyl)-L-glutamamide (23a) and N^5-hydroxy-N^1-methyl-N^2-(4-methylbenzoyl)-L-glutamamide (2b)

To a stirred solution of **2a** (257 mg, 0.924 mmol) in DMF (1 mL) were added *O*-benzylhydroxylamine hydrochloride (177 mg, 1.11 mmol), 1-hydroxybenzotriazole monohydrate (170 mg, 1.11 mmol), triethylamine (0.155 mL, 1.11 mmol), and 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (213 mg, 1.11 mmol) at 0 °C. After stirring at room temperature for 1 h, the reaction mixture was diluted with EtOAc. The resulting suspension was washed with 1 N HCl, saturated $NaHCO_3$, and brine, and dried over $MgSO_4$. Removal of the volatiles in vacuo provided **23a** (205 mg, 58% yield) as a white powder. Compound **23a**: TLC R_f = 0.44 ($CHCl_3$–MeOH, 9:1). Catalytic hydrogenation of **23a** (205 mg, 0.699 mmol) in a mixed solvent of MeOH–$CHCl_3$ (2:1, 3 mL) was conducted at room temperature in the presence of 5% palladium on carbon (22 mg) at atmospheric pressure. Removal of the catalyst by filtration followed by evaporation of the solvent afforded a solid. The resulting solid was purified by silica gel chromatography with a gradient from MeOH–$CHCl_3$ (3:97) to MeOH–$CHCl_3$ (1:9) to give **2b** (77 mg, 49% yield) as a white amorphous powder. Compound **2b**: TLC R_f = 0.69 ($CHCl_3$–MeOH, 4:1); MS (FAB, pos.) m/z 294 $(M+H)^+$; IR (KBr) 3267, 1636, 1538, 1189, 838, 753, 433 cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6): δ 10.37 (br s, 1H), 8.69 (m, 1H), 8.43 (d, J = 7.6 Hz, 1H), 7.85–7.79 (m, 3H), 7.25 (d, J = 8.0 Hz, 2H), 4.40–4.25 (m, 1H), 2.59 (d, J = 4.6 Hz, 3H), 2.06 (s, 3H), 2.10–1.85 (m, 4H); optical rotation $[\alpha]_D^{30}$ +7.96° (c 0.515, MeOH); HRMS (FAB) calcd for $C_{14}H_{20}N_3O_4$: 294.1454. Found: 294.1457.

5.7. N^5-(Benzyloxy)-N^1-methyl-N^2-(4-methylbenzoyl)-D-glutamamide (23b) and N^5-hydroxy-N^1-methyl-N^2-(4-methylbenzoyl)-D-glutamamide (5)

The hydroxamate **23b** was obtained as a white powder in 61% yield from **22** according to the analogous procedures as described for the preparation of **23a**. Compound **23b**: TLC R_f = 0.69 ($CHCl_3$–MeOH, 5:1). The hydroxamic acid **5** was obtained as a white powder in 94% yield from **23b** according to the analogous procedures as described for the preparation of **2b**. Compound **2b**: TLC R_f = 0.30 ($CHCl_3$–MeOH, 10:1); MS (APCI, neg. 20 V) m/z 292 $(M-H)^-$; IR (KBr) 3322, 1652, 1615, 1537, 1506, 1449, 1413, 1384, 1302, 1257, 1184, 1075, 1031, 834, 752, 686, 600 cm^{-1}; ^{1}H NMR (200 MHz, CD_3OD): δ 7.72 (d, J = 8.0 Hz, 2H), 7.20 (d, J = 8.0 Hz, 2H), 4.50–4.44 (m, 1H), 2.67 (s, 3H), 2.31 (s, 3H), 2.22–1.85 (m, 4H); optical rotation $[\alpha]_D^{31}$ −5.76° (c 0.545, MeOH); HRMS (FAB) calcd for $C_{14}H_{20}N_3O_4$: 294.1454. Found: 294.1456.

5.8. Benzyl benzyloxycarbamate (25)

To a stirred suspension of **24** (16.0 g, 100 mmol) in CH_2Cl_2 (100 mL) were added *N*,*N*-(diisopropyl)ethyl-

amine (34.8 mL, 200 mmol) and benzyl chloroformate (14.3 mL, 100 mmol) at 0 °C. The reaction mixture was stirred at 0 °C for 2 h and diluted with EtOAc. The resulting suspension was washed with 2 N HCl, and brine, and dried over $MgSO_4$. Removal of the volatiles in vacuo provided an oily solid, which was triturated with *n*-hexane to give **25** (21.9 g, 85% yield) as a white powder: TLC $R_f = 0.24$ (EtOAc–*n*-hexane, 1:4); ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.35 (m, 10H), 5.18 (s, 2H), 4.87 (s, 2H).

5.9. 5-{(Benzyloxy)[(benzyloxy)carbonyl]amino}-5-oxopentanoic acid (26a) and benzyl *N*-[5-(methylamino)-5-oxopentanoyl]benzyloxycarbamate (26b)

To a stirred solution of glutaric anhydride (1.26 g, 11.0 mmol) in acetonitrile (10 mL) were added **25** (2.57 g, 10.0 mmol) and 4-(dimethylamino)pyridine (12 mg, 1.0 mmol) at room temperature. The mixture was stirred for 40 h at room temperature. Glutaric anhydride (1.26 g, 11.0 mmol) and 4-(dimethylamino)pyridine (50 mg, 4.2 mmol) were added to it. The mixture was stirred for 3 h at room temperature, and concentrated under reduced pressure. The residue was acidified with 1 N HCl, and extracted with EtOAc. The extract was washed with brine, dried over $MgSO_4$, and filtrated. Concentration under reduced pressure gave a white powder, which was triturated with hexane to afford **26a** as a white powder (4.57 g): TLC $R_f = 0.67$ ($CHCl_3$–MeOH–AcOH, 18:2:1). To a stirred mixture of **26a** (1.11 g, 2.99 mmol), methylamine (40% in MeOH, 0.50 g, 5.98 mmol), *N*,*N*-(diisopropyl)ethylamine (1.56 mL, 8.97 mmol), and HOBt (686 mg, 4.48 mmol) in DMF (5 mL) was added EDC·HCl (859 mg, 4.48 mmol) at 0 °C. The mixture was stirred for 64 h at an ambient temperature, and diluted with EtOAc. The suspension was washed with brine, 1 N HCl, brine, satd $NaHCO_3$, and brine, and dried over $MgSO_4$. Removal of the volatiles in vacuo provided a white powder, which was triturated with Et_2O to give **26b** (68 mg, 7% yield from **25**) as a colorless silky powder: ^{1}H NMR ($CDCl_3$, 200 MHz): δ 7.41 (m, 5H), 7.34 (m, 5H), 5.54 (m, 1H), 5.27 (s, 2H), 4.90 (s, 2H), 2.85 (t, $J = 7.0$ Hz, 2H), 2.80 (d, $J = 5.2$ Hz, 3H), 2.24 (t, $J = 6.6$ Hz, 2H), 2.02 (m, 2H).

5.10. *N*-Hydroxy-*N*′-methylpentanediamide (3)

The title compound was obtained as colorless crystals in 81% yield from **26** according to the analogous procedures as described for the preparation of **2b**. TLC $R_f = 0.61$ (EtOAc–AcOH–H_2O, 3:1:1); MS (APCI, neg. 40 V) *m*/*z* 159 $(M-H)^-$; IR (KBr) 3224, 2942, 1646, 1562, 1459, 1413, 1291, 1165, 1092, 1071, 1023, 604, 439 cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6): δ 10.30–9.30 (br, 1H), 9.30–8.30 (m, 1H), 7.69 (m, 1H), 2.55 (d, $J = 4.8$ Hz, 3H), 2.05 (t, $J = 7.3$Hz, 2H), 1.94 (t, $J = 7.7$ Hz, 2H), 1.70 (tt, $J = 7.7$, 7.3 Hz, 2H); HRMS (FAB) calcd for $C_6H_{13}N_2O_3$: 161.0926. Found 161.0926.

5.11. 4-[(4-Methylbenzoyl)amino]butanoic acid (28)

To a stirred suspension of 4-aminobutyric acid (1.13 g, 11.0 mmol) in THF (21 mL) were added 1 N NaOH (21 mL, 21 mmol) at 0 °C and 4-methylbenzoyl chloride **27** (1.55 g, 10.0 mmol). After stirring at room temperature for 3 h, the reaction mixture was acidified with 2 N HCl and extracted with EtOAc. The organic layer was washed with brine and dried over $MgSO_4$. Removal of the volatiles in vacuo provided a solid, which was washed with diethyl ether to give **28** (2.00 g, 90% yield) as colorless crystals: TLC $R_f = 0.58$ ($CHCl_3$–MeOH–AcOH, 18:2:1); ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.73 (d, $J = 8.4$ Hz, 2H), 7.40 (m, 1H), 7.21 (d, $J = 8.4$ Hz, 2H), 3.47 (m, 2H), 2.41 (t, $J = 7.3$ Hz, 2H), 2.38 (s, 3H), 1.94 (m, 2H).

5.12. *N*-{4-[(Benzyloxy)amino]-4-oxobutyl}-4-methylbenzamide (29)

The title compound was obtained as a white powder in 76% yield from **28** according to the analogous procedures as described for the preparation of **23a**: TLC $R_f = 0.52$ ($CHCl_3$–MeOH, 10:1); ^{1}H NMR (200 MHz, $CDCl_3$): δ 9.24 (s, 1H), 7.68 (d, $J = 8.2$ Hz, 2H), 7.37 (m, 5H), 7.23 (d, $J = 8.2$ Hz, 2H), 6.66 (m, 1H), 4.90 (s, 2H), 3.46 (dt, $J = 6.0$, 5.8 Hz, 2H), 2.39 (s, 3H), 2.17 (m, 2H), 1.92 (m, 2H).

5.13. *N*-(4-Hydroxyamino-4-oxobutyl)-4-methylbenzamide (4)

The title compound was obtained as colorless crystals in 85% yield from **29** according to the analogous procedures as described for the preparation of **2b**: TLC $R_f = 0.23$ ($CHCl_3$–MeOH, 10:1); MS (APCI, neg. 40 V) *m*/*z* 235 $(M-H)^-$; IR (KBr) 1674, 1613, 1608, 1564, 1510, 1458, 1436, 1360, 1332, 1313, 1258, 1233, 1197, 1178, 1101, 1024, 969 cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6): δ 10.39 (s, 1H), 8.70 (s, 1H), 8.40 (t, $J = 5.2$ Hz, 1H), 7.74 (d, $J = 8.1$ Hz, 2H), 7.25 (d, $J = 8.1$ Hz, 2H), 3.24 (td, $J = 6.6$, 5.2 Hz, 2H), 2.35 (s, 3H), 2.02 (t, $J = 7.7$ Hz, 2H), 1.74 (m, 2H); HRMS (FAB) calcd for $C_{12}H_{17}N_2O_3$: 237.1239. Found 237.1244.

5.14. Benzyl N^2-(*tert*-butoxycarbonyl)-N^1-(4-methylphenyl)-D-glutaminate (30)

To a stirred solution of **19b** (6.75 g, 20.0 mmol) in DMF (20 mL) were added *p*-methylaniline (4.31 g, 30.0 mmol), triethylamine (8.36 mL, 60.0 mmol), 1-hydroxybenzotriazole monohydrate (3.67 g, 24.0 mmol), and 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (4.60 g, 24.0 mmol) at 0 °C. The reaction mixture was stirred at room temperature for 16 h. The solution was diluted with EtOAc, and washed sequentially with 1N HCl, saturated $NaHCO_3$, and brine. The organic layer was dried over $MgSO_4$. Removal of the volatiles in vacuo provided brown oil, which was recrystallized from EtOAc–*n*-hexane (1:4) to give **30** (8.14 g, 98% yield) as colorless crystals: TLC $R_f = 0.54$ (EtOAc–*n*-hexane, 2:3); ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.30 (br s, 1H), 7.35 (m, 7H), 7.11 (d, $J = 8.0$ Hz, 2H), 5.38 (d, $J = 8.0$ Hz, 1H), 5.16 (d, $J = 13.0$ Hz, 1H), 5.13 (d, $J = 13.0$ Hz, 1H), 4.28 (m, 1H), 2.62 (m, 1H), 2.50 (m, 1H), 2.31 (s, 3H), 2.22 (m, 1H), 2.02 (m, 1H), 1.44 (s, 9H).

5.15. N^2-(*tert*-Butoxycarbonyl)-N^1-(4-methylphenyl)-D-α-glutamine (31)

The title compound was obtained quantitatively as a colorless oil from **30** according to the analogous procedures as described for the preparation of **22**: TLC $R_f = 0.57$ ($CHCl_3$–MeOH–AcOH, 18:2:1).

5.16. N^2-(*tert*-Butoxycarbonyl)-N^5-benzyloxy-N^1-(4-methylphenyl)-D-glutamamide (32)

The title compound was obtained as a white powder in 98% yield from **31** according to the analogous procedures as described for the preparation of **23a**: TLC $R_f = 0.41$ ($CHCl_3$–MeOH, 10:1).

5.17. N^5-Benzyloxy-N^1-(4-methylphenyl)-D-glutamamide (33)

A solution of **32** (3.25 g, 7.56 mmol) in 4 N HCl in 1,4-dioxane (20 mL) was stirred at room temperature for 3 h. The reaction mixture was evaporated to give an oily residue, which was triturated with Et_2O to give **33** (2.74 g, 96% yield) as a white powder: TLC $R_f = 0.24$ ($CHCl_3$–MeOH–AcOH, 18:2:1).

5.18. N^2-Acetyl-N^5-benzyloxy-N^1-(4-methylphenyl)-D-glutamamide (34)

To a stirred solution of **33** (378 mg, 1.00 mmol) in CH_2Cl_2 (5 mL) were added *N*,*N*-(diisopropyl)ethylamine (0.38 mL, 2.2 mmol) and acetic anhydride (0.094 mL, 1.0 mmol) at 0 °C. The reaction mixture was stirred at room temperature for 16 h. The mixture was concentrated under reduced pressure to give a powder, which was washed with H_2O, 1 N HCl, H_2O, saturated $NaHCO_3$, H_2O, and dried under reduced pressure. The obtained powder was dissolved in MeOH. The solution was evaporated to give a solid, which was triturated with Et_2O to give **34** (320 mg, 83% yield) as a white powder: TLC $R_f = 0.51$ ($CHCl_3$–MeOH, 10:1).

5.19. N^2-Acetyl-N^5-hydroxy-N^1-(4-methylphenyl)-D-glutamamide (6)

The title compound was obtained as a white powder in 89% yield from **34** according to the analogous procedures as described for the preparation of **2b**: TLC $R_f = 0.13$ ($CHCl_3$–MeOH, 10:1); MS (APCI, neg. 40 V) *m*/*z*, 292 $(M-H)^-$; IR (KBr) 3269, 2900, 1657, 1607, 1538, 1450, 1381, 1287, 1257, 1109, 1086, 1021, 981, 818, 797, 686, 610, 514 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6): δ 10.41 (s, 1H), 9.94 (s, 1H), 8.70 (s, 1H), 8.16 (d, $J = 7.8$ Hz, 1H), 7.48 (d, $J = 8.4$ Hz, 2H), 7.10 (d, $J = 8.4$ Hz, 2H), 4.37 (m, 1H), 2.25 (s, 3H), 2.00 (m, 2H), 1.87 (s, 3H), 1.95–1.70 (m, 2H); optical rotation $[\alpha]_D^{31}$ +18.2° (*c* 0.50, MeOH); HRMS (FAB) calcd for $C_{14}H_{20}N_3O_4$: 294.1454. Found: 294.1453.

5.20. 5-Benzyl 1-*tert*-butyl *N*-(4-methylbenzoyl)-L-glutamate (36)

To a stirred solution of **35** (10.0 g, 30.3 mmol) in $CHCl_3$ (50 mL) were added *N*,*N*-(diisopropyl)ethylamine (11.6 mL, 66.7 mmol) and 4-methylbenzoyl chloride (4.41 mL, 33.4 mmol) at 0 °C. After stirring at room temperature for 1 h, saturated $NaHCO_3$ was added to the reaction mixture with vigorous stirring. After stirring for 30 min, the reaction mixture was then diluted with EtOAc and washed sequentially with brine, 1 N HCl, brine, saturated $NaHCO_3$, and brine. The organic layer was dried over $MgSO_4$. Removal of the volatiles in vacuo provided a solid, which was triturated with *n*-hexane to give **36** (12.6 g, quantitative yield) as a white powder: TLC $R_f = 0.19$ (EtOAc–*n*-hexane, 1:4).

5.21. 1-*tert*-Butyl *N*-(4-methylbenzoyl)-L-glutamate (37)

The title compound was obtained as colorless crystals from **36** in 76% yield according to the analogous procedures as described for the preparation of **22**: ^1H NMR (200 MHz, $CDCl_3$): δ 7.70 (d, $J = 8.2$ Hz, 2H), 7.22 (d, $J = 8.2$ Hz, 2H), 6.97 (d, $J = 7.5$ Hz, 1H), 4.71 (m, 1H), 2.47 (m, 2H), 2.38 (s, 3H), 2.28 (m, 1H), 2.05 (m, 1H), 1.49 (s, 9H).

5.22. *tert*-Butyl N^5-benzyloxy-N^5-[(benzyloxy)carbonyl]-N^2-(4-methylbenzoyl)-L-α-glutaminate (38)

To a stirred solution of **37** (643 mg, 2.00 mmol) in DMF (10 mL) were added **25** (515 mg, 2.00 mmol), 4-(dimethylamino)pyridine (24 mg, 0.20 mmol), and 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (422 mg, 2.20 mmol) at 0 °C. The mixture was stirred at room temperature for 16 h, and diluted with EtOAc. The suspension was washed with brine, 1 N HCl, brine, saturated $NaHCO_3$, brine, dried over $MgSO_4$. Removal of the volatiles in vacuo provided a colorless oil, which was purified by silica gel chromatography with EtOAc–*n*-hexane (3:7) to give **38** (992 mg, 88% yield) as a colorless syrup: TLC $R_f = 0.27$ (EtOAc–*n*-hexane, 3:7); ^1H NMR (200 MHz, $CDCl_3$): δ 7.72 (d, $J = 8.0$ Hz, 2H), 7.38 (m, 5H), 7.30 (m, 5H), 7.20 (d, $J = 8.0$ Hz, 2H), 6.89 (d, $J = 7.8$ Hz, 1H), 5.25 (s, 2H), 4.89 (d, $J = 10.0$ Hz, 1H), 4.85 (d, $J = 10.0$ Hz, 1H), 4.72 (m, 1H), 2.94 (m, 2H), 2.38 (s, 3H), 2.30 (m, 1H), 2.16 (m, 1H), 1.49 (s, 9H); optical rotation $[\alpha]_D^{25}$ +6.84° (*c* 0.655, $CHCl_3$).

5.23. N^5-Benzyloxy-N^5-[(benzyloxy)carbonyl]-N^2-(4-methylbenzoyl)-L-glutamine (39)

A solution of **38** (980 mg, 1.75 mmol) in trifluoroacetic acid (5 mL) was stirred at room temperature for 3 h. The reaction mixture was evaporated to give a crude oil, which was purified by silica gel chromatography with AcOH–MeOH–$CHCl_3$ (5:10:85) to give a solid, which was triturated with diisopropylether to give **39** (495 mg, 56% yield) as a white powder: TLC $R_f = 0.30$ ($CHCl_3$–MeOH–AcOH, 18:2:1).

5.24. N^5-Hydroxy-N^2-(4-methylbenzoyl)-L-glutamine (7)

The title compound was obtained as a white powder in 95% yield from **39** according to the analogous procedures as described for the preparation of **2b**: TLC $R_f = 0.43$ (EtOAc–AcOH–H_2O, 8:1:1); MS (APCI, neg.

40 V) *m/z* 279 $(M-H)^-$; IR (KBr) 3240, 3037, 2978, 2925, 1719, 1636, 1543, 1505, 1452, 1409, 1340, 1292, 1242, 1191, 1104, 1021, 979, 839, 755, 579 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6): δ 10.47 (m, 1H), 8.49 (d, J = 7.4 Hz, 1H), 7.79 (d, J = 7.8 Hz, 2H), 7.27 (d, J = 7.8 Hz, 2H), 4.30 (m, 1H), 2.36 (s, 3H), 1.80–2.20 (m, 4H); optical rotation $[\alpha]_D^{31}$ +1.30° (*c* 0.50, MeOH); HRMS (FAB) calcd for $C_{13}H_{17}N_2O_5$: 281.1137. Found: 281.1143.

5.25. N^5-Benzyloxy-N^1-(4-hydroxybutyl)-N^2-(4-methylbenzoyl)-L-glutamamide (40)

To a stirred solution of **39** (140 mg, 0.278 mmol) in DMF (1 mL) were added 4-amino-1-butanol (37 mg, 0.42 mmol), 1-hydroxybenzotriazole monohydrate (65 mg, 0.42 mmol), and 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (80 mg, 0.42 mmol) at room temperature. The reaction mixture was stirred at room temperature for 16 h. The solution was then diluted with EtOAc. The mixture was washed sequentially with saturated NH_4Cl, saturated $NaHCO_3$, brine, and dried over $MgSO_4$. Removal of the volatiles in vacuo provided **40** (69 mg, 57% yield) as a white powder: TLC R_f = 0.61 ($CHCl_3$–MeOH–AcOH, 18:2:1); MS (APCI, neg. 20 V) *m/z* 440 $(M-H)^-$; ^1H NMR (200 MHz, $CDCl_3$): δ 10.85 (br s, 1H), 10.37 (br s, 1H), 7.71 (d, J = 8.1 Hz, 2H), 7.40–7.23 (m, 5H), 7.18 (d, J = 8.1 Hz, 2H), 7.00–6.95 (m, 1H), 4.86 (s, 2H), 4.65–4.50 (m, 1H), 3.56–3.04 (m, 5H), 2.55–1.95 (m, 7H), 1.65–1.43 (m, 4H).

5.26. N^5-Hydroxy-N^1-(4-hydroxybutyl)-N^2-(4-methylbenzoyl)-L-glutamamide (8)

The title compound was obtained as a white powder in 94% yield from **40** according to the analogous procedures as described for the preparation of **2b**: TLC R_f = 0.23 ($CHCl_3$–MeOH–AcOH, 18:2:1); MS (APCI, neg. 20 V) *m/z* 350 $(M-H)^-$; IR (KBr) 3277, 2929, 1641, 1543, 1502, 1450, 1377, 1322, 1189, 1054, 753 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ 7.74 (d, J = 7.5 Hz, 2H), 7.39 (s, 1H), 7.25 (d, J = 7.5 Hz, 2H), 4.60–4.43 (m, 1H), 3.61–3.56 (m, 2H), 3.31–3.15 (m, 2H), 2.40 (s, 3H), 2.30–2.04 (m, 4H), 1.62–1.48 (m, 4H); optical rotation $[\alpha]_D^{31}$ +2.67° (*c* 0.525, MeOH); HRMS (FAB) calcd for $C_{17}H_{26}N_3O_5$: 352.1872. Found: 352.1872.

5.27. Methyl (4*S*)-4-{[(benzyloxy)carbonyl]amino}-5-hydroxypentanoate (42)

To a stirred solution of 5-methyl *N*-carbobenzoxy-L-glutamate **41** (45.7 g, 155 mmol) in THF (250 mL) were added *N*-hydroxysuccinimide (20.5 g, 178 mmol) and *N*,*N*-(dicyclohexyl)carbodiimide (36.8 g, 178 mmol) at 0 °C. The reaction mixture was stirred overnight. The resulting insoluble substance was removed by filtration. To the filtrate was added $NaBH_4$ (8.80 g, 233 mmol) at 0 °C. After stirring for 2 h at 0 °C, the reaction mixture was quenched with 2 N HCl and extracted with EtOAc. The organic layer was washed with 1 N HCl, and brine, and dried over $MgSO_4$. Removal of the volatiles in vacuo provided a solid, which purified by silica gel chromatography with *n*-hexane– EtOAc (2:3) to give **42** (31.4 g, 72% yield) as a white powder: TLC R_f = 0.35 (*n*-hexane–EtOAc, 2:3); ^1H NMR (200 MHz, $CDCl_3$ + two drops of CD_3OD): δ 7.45–7.20 (m, 5H), 5.48 (d, J = 9.0 Hz, 1H), 5.09 (s, 2H), 3.80–3.50 (m, 6H), 2.42 (t, J = 7.4 Hz, 2H), 2.00–1.70 (m, 2H).

5.28. Methyl (4*S*)-4-{[(benzyloxy)carbonyl]amino}-5-(ethoxymethoxy)pentanoate (43)

To a stirred solution of **42** (12.0 g, 42.7 mmol) in CH_2Cl_2 (45 mL) were added *N*,*N*-(diisopropyl)ethylamine (22 mL) and chloromethyl ethyl ether (7.95 mL, 85.4 mmol) at 0 °C. After stirring at room temperature for 80 min, the solution was poured into 1 N HCl and extracted with EtOAc. The organic layer was washed with saturated $NaHCO_3$, and brine, and dried over Na_2SO_4. Removal of the volatiles in vacuo provided an oily residue, which was purified by silica gel chromatography with EtOAc–*n*-hexane (1:2) to give **43** (10.4 g, 72% yield) as a colorless oil: TLC R_f = 0.61 (EtOAc–*n*-hexane, 1:1); MS (MALDI, pos.) *m/z* 378 $(M+K)^+$, 362 $(M+Na)^+$, 340 $(M+H)^+$; ^1H NMR (200 MHz, $CDCl_3$): δ 7.37–7.28 (m, 5H), 5.10 (s, 2H), 5.05 (d, J = 9.0 Hz, 1H), 4.65 (s, 2H), 3.90–3.78 (m, 1H), 3.65 (s, 3H), 3.61–3.52 (m, 4H), 2.41 (t, J = 7.6 Hz, 2H), 1.94–1.84 (m, 2H), 1.20 (t, J = 7.1 Hz, 3H).

5.29. Methyl (4*S*)-5-ethoxymethoxy-4-[(4-methylbenzoyl)-amino]pentanoate (44)

Catalytic hydrogenation of **43** (3.39 g, 10.0 mmol) in MeOH (30 mL) was conducted at room temperature for 1 h in the presence of 10% palladium on carbon (600 mg) at atmospheric pressure. Removal of the catalyst by filtration followed by evaporation afforded the amine. To a stirred solution of the above amine in CH_2Cl_2 (30 mL) were added triethylamine (1.8 mL, 13 mmol) and 4-methylbenzoyl chloride (1.5 mL, 11 mmol) at 0 °C. After stirring at room temperature overnight, the reaction mixture was diluted with EtOAc. The resulting solution was washed with 1 N HCl, saturated $NaHCO_3$, and brine, and dried over $MgSO_4$. Removal of the volatiles in vacuo provided an oily residue, which was purified by silica gel chromatography with a gradient from EtOAc–*n*-hexane (1:3) to EtOAc–*n*-hexane (2:3) to give **44** (2.47 g, 76% yield) as a white powder: TLC R_f = 0.30 (EtOAc–*n*-hexane, 1:1); MS (MALDI, pos.) *m/z* 324 $(M+H)^+$.

5.30. (4*S*)-5-Ethoxymethoxy-4-[(4-methylbenzoyl)amino]pentanoic acid (45)

To a stirred solution of **44** (751 mg, 2.33 mmol) in MeOH (5 mL) at room temperature was added 2 N NaOH (2.3 mL, 4.6 mmol). The reaction mixture was stirred at room temperature for 2 h. The mixture was neutralized with 2 N HCl and extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated. The resulting residue was triturated with Et_2O–*n*-hexane to give **45** (510 mg, 72% yield) as a white powder; MS (APCI, neg. 20 V) *m/z* 308 $(M-H)^-$; ^1H NMR (200 MHz, DMSO-d_6): δ

12.01 (s, 1H), 8.05 (d, $J = 8.4$ Hz, 1H), 7.72 (d, $J = 8.1$ Hz, 2H), 7.23 (d, $J = 8.1$ Hz, 2H), 4.57 (s, 2H), 4.17–3.99 (m, 1H), 3.54–3.39 (m, 4H), 2.32 (s, 3H), 2.23 (t, $J = 7.4$ Hz, 2H), 1.94–1.57 (m, 1H), 1.07 (t, $J = 7.0$ Hz, 3H).

5.31. *N*-[(1*S*)-1-[(Ethoxymethoxy)methyl]-4-(hydroxyamino)-4-oxobutyl]-4-methylbenzamide (9)

To a stirred solution of **45** (500 mg, 1.62 mmol) in DMF (10 mL) were added 1-hydroxybenzotriazole monohydrate (500 mg, 3.24 mmol), 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (620 mg, 3.24 mmol) and *O*-(2-methoxy-2-propyl)hydroxylamine (340 mg, 3.24 mmol) at room temperature. After stirring at room temperature for 3 h, the reaction mixture was diluted with water, and extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated. The residue was purified by silica gel chromatography with a gradient from $CHCl_3$ to MeOH–$CHCl_3$ (2:98) to give a solid, which was dissolved in MeOH (3 mL). The solution was acidified with 1 N HCl to pH 3. After stirring at room temperature for 10 min, the solution was concentrated in vacuo. The residue was purified by silica gel chromatography with a gradient from $CHCl_3$ to MeOH–$CHCl_3$ (4:96) to give **9** (96 mg, 18% yield) as a light brown amorphous powder: TLC $R_f = 0.30$ ($CHCl_3$–MeOH, 9:1); MS (APCI, neg. 40 V) *m*/*z* 323 $(M-H)^-$; IR (KBr) 3236, 2976, 2928, 2878, 1636, 1541, 1506, 1454, 1101, 1038, 838, 753, 666, 602 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 10.32 (s, 1H), 8.65 (s, 1H), 8.10 (d, $J = 8.4$ Hz, 1H), 7.73 (d, $J = 8.3$ Hz, 2H), 7.22 (d, $J = 8.3$ Hz, 2H), 4.57 (s, 2H), 4.10–3.98 (m, 1H), 3.52–3.40 (m, 4H), 2.32 (s, 3H), 2.01–1.94 (m, 1H), 1.91–1.78 (m, 1H), 1.73–1.61 (m, 1H), 1.06 (t, $J = 7.1$ Hz, 3H); optical rotation $[\alpha]_D^{30}$ −23.85° (*c* 0.535, MeOH); HRMS (FAB) calcd for $C_{16}H_{25}N_2O_5$: 325.1763. Found: 325.1761.

5.32. 2-Benzyloxymethyl-4-[(4-methylbenzoyl)amino]-butanoic acid (46)

To a stirred solution of *N*,*N*-(diisopropyl)ethylamine (0.925 mL, 6.60 mmol) in a mixed solvent of dry THF (5 mL) and HMPA (3 mL) was added 4.05 mL of 1.63 M *n*-BuLi in hexane (6.60 mmol) at −78 °C under argon. The mixture was stirred at −78 °C for 15 min. A solution of 4-[(4-methylbenzoyl)amino]butanoic acid **28** (442 mg, 2.00 mmol) in dry THF (3 mL) was added to the above described solution at −78 °C. The reaction mixture was allowed to warm up to room temperature for 30 min. The reaction mixture was cooled at −78 °C. Benzyloxymethyl chloride (313 mg, 2.00 mmol) was added to the mixture at −78 °C. Stirring was continued at −78 °C for 2 h followed by the addition of 1 N HCl. The reaction mixture was extracted with EtOAc, washed with brine, and dried over $MgSO_4$. Removal of the volatiles in vacuo provided **46** (944 mg, quantitative yield) as a yellow oil. This compound was used for the next reaction without further purification: TLC $R_f = 0.67$ ($CHCl_3$–MeOH–AcOH, 18:2:1); MS (MALDI, pos.) *m*/*z* 364 $(M+Na)^+$, 342 $(M+H)^+$.

5.33. *N*-(4-Benzyloxyamino-3-benzyloxymethyl-4-oxobutyl)-4-methylbenzamide (47)

The title compound was obtained as a white powder in 31% yield from **46** according to the analogous procedures as described for the preparation of **23a**: TLC $R_f = 0.17$ (EtOAc–*n*-hexane, 3:2); MS (MALDI, pos.) *m*/*z* 485 $(M+K)^+$, 469 $(M+Na)^+$, 447 $(M+H)^+$; ^1H NMR (200 MHz, $CDCl_3$): δ 9.57 (s, 1H), 7.66 (d, $J = 8.1$ Hz, 2H), 7.50–7.10 (m, 12H), 6.67 (m, 1H), 4.88 (s, 2H), 4.45 (d, $J = 12.1$ Hz, 1H), 4.40 (d, $J = 12.1$ Hz, 1H), 3.49 (m, 4H), 2.47 (m, 1H), 2.38 (s, 3H), 1.85 (m, 2H).

5.34. *N*-(3-Benzyloxymethyl-4-hydroxyamino-4-oxobutyl)-4-methylbenzamide (13)

The title compound was obtained as a white powder in 82% yield from **47** according to the analogous procedures as described for the preparation of **2b**: TLC $R_f = 0.36$ ($CHCl_3$–MeOH, 10:1); MS (MALDI, pos.) *m*/*z* 395 $(M+K)^+$, 379 $(M+Na)^+$, 357 $(M+H)^+$; IR (KBr) 3249, 3063, 3031, 2922, 2868, 1663, 1636, 1549, 1507, 1455, 1365, 1310, 1206, 1190, 1103, 1077, 1029, 932, 838, 751, 699, 636, 613 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6): δ 10.52 (s, 1H), 8.86 (s, 1H), 8.32 (t, $J = 5.5$ Hz, 1H), 7.74 (d, $J = 8.4$ Hz, 2H), 7.31 (m, 5H), 7.24 (d, $J = 8.4$ Hz, 2H), 4.46 (d, $J = 12.5$ Hz, 1H), 4.44 (d, $J = 12.5$ Hz, 1H), 3.59 (t, $J = 8.8$ Hz, 1H), 3.41 (dd, $J = 8.8$, 5.5 Hz, 1H), 3.21 (m, 2H), 2.44 (m, 1H), 2.35 (s, 3H), 1.66 (m, 2H); HRMS (FAB) calcd for $C_{20}H_{25}N_2O_4$: 357.1814. Found: 357.1818.

5.35. *N*-(4-Hydroxyamino-3-hydroxymethyl-4-oxobutyl)-4-methylbenzamide (10)

To a stirred suspension of **13** (100 mg, 0.28 mmol) in CH_2Cl_2 (10 mL) was added 0.98 mL of 1.0 M BBr_3 in CH_2Cl_2 (0.98 mmol) at 0 °C under argon. The suspension was stirred at an ambient temperature for 2 h, and quenched with 1 N HCl. The reaction mixture was washed with EtOAc. The aqueous layer was concentrated under reduced pressure. The residue was dissolved in H_2O (10 mL). Silica gel (5 g) was added to the solution, which was concentrated under reduced pressure and dried in vacuo. The powder was bedded on silica gel chromatography. Elution with AcOH–H_2O–EtOAc (2:3:16) provided a crude oil, which was triturated with Et_2O to give **10** (54 mg, 72% yield) as a beige powder: TLC $R_f = 0.39$ (EtOAc–AcOH–H_2O, 16:3:2); MS (MALDI, pos.) *m*/*z* 289 $(M+Na)^+$; IR (KBr) 3279, 2932, 2879, 1636, 1549, 1507, 1439, 1408, 1385, 1323, 1310, 1191, 1155, 1119, 1022, 927, 865, 753, 661, 636 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6): δ 8.41 (m, 1H), 7.76 (d, $J = 8.0$ Hz, 2H), 7.24 (d, $J = 8.0$ Hz, 2H), 3.52 (m, 1H), 3.37 (m, 1H), 3.20 (m, 2H), 2.35 (s, 3H), 2.23 (m, 1H), 1.64 (m, 2H); HRMS (FAB) calcd for $C_{13}H_{19}N_2O_4$: 267.1345. Found: 267.1353.

5.36. (+)-*N*-(4-Hydroxyamino-3-hydroxymethyl-4-oxobutyl)-4-methylbenzamide (11) and (−)-*N*-(4-hydroxyamino-3-hydroxymethyl-4-oxobutyl)-4-methylbenzamide (12)

Compound **47** was separated into two fractions by HPLC using chiral column ($t_R = 13.66$ and 14.90 min, DAICEL

CHIRALPAK AD, 20% isopropanol in *n*-hexane, 0.80 mL/min, 256 nm). The fraction with retention time of 13.66 min was concentrated under reduced pressure to give an oily residue. The residue was treated with 5% palladium on carbon under hydrogen atmosphere by the analogous procedures described for the preparation of **2b** to yield **11** as a brown powder. Compound **11**: TLC $R_f = 0.17$ ($CHCl_3$–MeOH, 4:1); MS (FAB, pos.) *m/z* 289 $(M+Na)^+$, 267 $(M+H)^+$; IR (KBr) 3289, 2925, 1640, 1544, 1505, 1309, 1190, 1120, 1035, 925, 837, 753 cm^{-1}; ^1H NMR (200 MHz, CD_3OD): δ 7.70 (d, $J = 8.2$ Hz, 2H), 7.26 (d, $J = 8.2$ Hz, 2H), 3.79–3.52 (m, 2H), 3.50–3.25 (m, 2H), 2.38 (s, 3H), 2.38–2.25 (m, 1H), 1.80 (m, 2H); optical rotation $[\alpha]_D^{27}$ +19.2° (*c* 0.14, MeOH); HRMS (FAB) calcd for $C_{13}H_{19}N_2O_4$: 267.1345. Found: 267.1339. The fraction with retention time of 14.90 min was concentrated under reduced pressure to give an oily residue. The residue was treated with 5% palladium on carbon under hydrogen atmosphere by the analogous procedures described for the preparation of **2b** to yield **12** as a brown powder. Compound **12**: TLC $R_f = 0.17$ ($CHCl_3$–MeOH, 4:1); MS (FAB, pos.) *m/z* 289 $(M+Na)^+$, 267 $(M+H)^+$; IR (KBr) 3289, 2925, 1640, 1544, 1505, 1309, 1190, 1120, 1035, 925, 837, 753 cm^{-1}; ^1H NMR (200 MHz, CD_3OD): δ 7.70 (d, $J = 8.2$ Hz, 2H), 7.26 (d, $J = 8.2$ Hz, 2H), 3.79–3.52 (m, 2H), 3.50–3.25 (m, 2H), 2.38 (s, 3H), 2.38–2.25 (m, 1H), 1.80 (m, 2H); optical rotation $[\alpha]_D^{29}$ −15.2° (*c* 0.165, MeOH); HRMS (FAB) calcd for $C_{13}H_{19}N_2O_4$: 267.1345. Found: 267.1346.

5.37. Ethyl 4-{[(*trans*-4-methylcyclohexyl)carbonyl]amino}butanoate (49a)

To a stirred solution of *trans*-4-methylcyclohexanecarboxylic acid **48a** (1.30 g, 9.14 mmol) in DMF (45 mL) were added ethyl 4-aminobutanoate hydrochloride (1.99 g, 11.9 mmol), 1-hydroxybenzotriazole monohydrate (1.68 g, 11.0 mmol), 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (2.10 g, 11.0 mmol), and triethylamine (1.5 mL, 11 mmol) at room temperature. The reaction mixture was stirred at room temperature overnight. The solution was then diluted with EtOAc and washed sequentially with saturated NH_4Cl, saturated $NaHCO_3$, and brine. The organic layer was dried over $MgSO_4$. Removal of the volatiles in vacuo provided a solid, which was purified by silica gel chromatography with EtOAc–AcOH–$CHCl_3$ (10:3:87) to give **49a** (1.44 g, 62% yield) as a white powder: TLC $R_f = 0.88$ ($CHCl_3$–MeOH–AcOH, 90:10:1); MS (MALDI, pos.) *m/z* 294 $(M+K)^+$, 278 $(M+Na)^+$, 256 $(M+H)^+$; ^1H NMR (200 MHz, $CDCl_3$): δ 5.70 (br s, 1H), 4.14 (q, $J = 7.2$ Hz, 2H), 3.29 (q, $J = 7.2$ Hz, 2H), 2.35 (t, $J = 7.1$ Hz, 2H), 2.07–1.71 (m, 7H), 1.56–1.22 (m, 7H), 1.05–0.84 (m, 4H).

5.38. Ethyl 4-{[(*trans*-4-propylcyclohexyl)carbonyl]amino}butanoate (49b)

The title compound was obtained as a white powder in 69% yield from *trans*-4-propylcyclohexanecarboxylic acid **48b** according to the analogous procedures as described for the preparation of **49a**: TLC $R_f = 0.20$ ($CHCl_3$–MeOH–AcOH, 90:10:1); MS (MALDI, pos.) *m/z* 322 $(M+K)^+$, 306 $(M+Na)^+$, 284 $(M+H)^+$; ^1H NMR (200 MHz, $CDCl_3$): δ 5.78–5.59 (br, 1H), 4.13 (q, $J = 7.1$ Hz, 2H), 3.28 (q, $J = 6.5$ Hz, 2H), 2.35 (t, $J = 7.1$ Hz, 2H), 2.08–1.75 (m, 7H), 1.54–1.10 (m, 10H), 1.01–0.84 (m, 5H).

5.39. Ethyl 4-[(1-benzofuran-2-ylcarbonyl)amino]butanoate (49c)

The title compound was obtained as a white solid in 69% yield from 1-benzofuran-2-carboxylic acid **48c** according to the analogous procedures as described for the preparation of **49a**: TLC $R_f = 0.20$ (*n*-hexane–EtOAc, 2:1); ^1H NMR (200 MHz, $CDCl_3$): δ 7.69–7.65 (m, 1H), 7.54–7.27 (m, 4H), 6.85 (m, 1H), 4.14 (q, $J = 7.4$ Hz, 2H), 3.55 (q, $J = 6.2$ Hz, 2H), 2.45 (t, $J = 7.0$ Hz, 2H), 2.07–1.96 (m, 2H), 1.25 (t, $J = 7.4$ Hz, 3H).

5.40. Ethyl 4-({5-[(4-methylphenyl)ethynyl]-2-furoyl}amino)butanoate (49d)

The title compound was obtained as a light yellow powder in 61% yield from 5-[(4-methylphenyl)ethynyl]-2-furoic acid **48d** according to the analogous procedures as described for the preparation of **49a**: TLC $R_f = 0.31$ ($CHCl_3$–MeOH–AcOH, 18:2:1); MS (MALDI, pos.) *m/z* 378 $(M+K)^+$, 362 $(M+Na)^+$, 340 $(M+H)^+$; ^1H NMR (200 MHz, $CDCl_3$): δ 7.44 (d, $J = 8.0$ Hz, 2H), 7.19 (d, $J = 8.0$ Hz, 2H), 7.12 (d, $J = 3.7$ Hz, 2H), 6.68 (d, $J = 3.7$ Hz, 1H), 6.64–6.53 (br, 1H), 4.14 (q, $J = 7.1$ Hz, 2H), 3.49 (q, $J = 6.6$ Hz, 2H), 2.46–2.36 (m, 5H), 1.96 (quintet, $J = 6.6$ Hz, 2H), 1.26 (t, $J = 7.1$ Hz, 3H).

5.41. Ethyl 4-{[5-(4-chlorophenyl)-2-furoyl]amino}butanoate (49e)

The title compound was obtained as an off-white powder in 60% yield from 5-(4-chlorophenyl)-2-furoic acid **48e** according to the analogous procedures as described for the preparation of **49a**: TLC $R_f = 0.80$ ($CHCl_3$–MeOH–AcOH, 18:2:1); MS (APCI, pos. 20 V) *m/z* 336 $(M+H)^+$; ^1H NMR (200 MHz, $CDCl_3$): δ 7.69 (d, $J = 8.8$ Hz, 2H), 7.40 (d, $J = 8.8$ Hz, 2H), 7.17 (d, $J = 3.6$ Hz, 1H), 6.88–6.78 (m, 1H), 6.72 (d, $J = 3.6$.Hz, 1H), 4.15 (q, $J = 7.1$ Hz, 2H), 3.52 (q, $J = 6.6$ Hz, 2H), 2.46 (t, $J = 6.6$ Hz, 2H), 1.99 (quintet, $J = 6.6$ Hz, 2H), 1.25 (t, $J = 7.1$ Hz, 3H).

5.42. 4-{[(*trans*-4-Methylcyclohexyl)carbonyl]amino}butanoic acid (50a)

To a stirred solution of **49a** (1.33 g, 5.22 mmol) in MeOH (8 mL) was added 1 N NaOH (6 mL, 6 mmol) at room temperature. The reaction mixture was stirred at room temperature for 8 h. The mixture was acidified with 1 N HCl and extracted with EtOAc. The organic layer was washed with brine and dried over $MgSO_4$. Removal of volatiles in vacuo provided **50a** (1.06 g, 90% yield) as a white powder: TLC $R_f = 0.55$ ($CHCl_3$–MeOH–AcOH, 18:2:1); MS (MALDI, pos.) *m/z* 250 $(M+Na)^+$, 228 $(M+H)^+$; ^1H NMR (200 MHz, DMSO-d_6): δ 12.01 (br s, 1H), 7.67 (t, $J = 6.0$ Hz, 1H), 3.02 (q, $J = 6.0$ Hz,

2H), 2.19 (t, $J = 7.5$ Hz, 2H), 2.06–1.91 (m, 1H), 1.74–1.52 (m, 6H), 1.46–1.18 (m, 4H), 0.98–0.76 (m, 4H).

5.43. 4-{[(*trans*-4-Propylcyclohexyl)carbonyl]amino}butanoic acid (50b)

The title compound was obtained as a white powder in 100% yield from **49b** according to the analogous procedures as described for the preparation of **50a**: TLC $R_f = 0.65$ ($CHCl_3$–MeOH–AcOH, 18:2:1); MS (MALDI, pos.) *m/z* 294 $(M+K)^+$, 278 $(M+Na)^+$, 256 $(M+H)^+$; ^1H NMR (200 MHz, DMSO-d_6): δ 12.00 (s, 1H), 7.74–7.61 (m, 1H), 3.02 (q, $J = 6.5$ Hz, 2H), 2.19 (t, $J = 7.2$ Hz, 2H), 2.11–1.92 (m, 1H), 1.78–1.53 (m, 6H), 1.43–1.08 (m, 7H), 0.95–0.89 (m, 5H).

5.44. 4-[(1-Benzofuran-2-ylcarbonyl)amino]butanoic acid (50c)

The title compound was obtained as a white powder in 65% yield from **49c** according to the analogous procedures as described for the preparation of **50a**: TLC $R_f = 0.32$ ($CHCl_3$–MeOH–AcOH, 100:10:1); ^1H NMR (200 MHz, DMSO-d_6): δ 8.73 (t, $J = 5.4$ Hz, 1H), 7.78–7.73 (m, 1H), 7.66–7.61 (m, 1H), 7.51 (d, $J = 0.8$ Hz, 1H), 7.49–7.41 (m, 1H), 7.36–7.28 (m, 1H), 3.28 (q, $J = 6.6$ Hz, 2H), 1.76 (t, $J = 7.4$ Hz, 2H), 1.83–1.68 (m, 2H).

5.45. 4-({5-[(4-Methylphenyl)ethynyl]-2-furoyl}amino)-butanoic acid (50d)

The title compound was obtained as a white solid in 100% yield from **49d** according to the analogous procedures as described for the preparation of **50a**: TLC $R_f = 0.54$ ($CHCl_3$–MeOH–AcOH, 18:2:1); MS (MALDI, pos.) *m/z* 350 $(M+K)^+$, 334 $(M+Na)^+$, 312 $(M+H)^+$; ^1H NMR (200 MHz, DMSO-d_6): δ 12.07 (br s, 1H), 8.51 (t, $J = 6.0$ Hz, 1H), 7.44 (d, $J = 8.1$ Hz, 2H), 7.25 (d, $J = 8.1$ Hz, 2H), 7.12 (d, $J = 3.7$ Hz, 1H), 6.94 (d, $J = 3.7$ Hz, 1H), 3.26–3.23 (m, 2H), 2.33 (s, 3H), 2.23 (d, $J = 7.5$ Hz, 2H), 1.79–1.63 (m, 2H).

5.46. 4-{[5-(4-Chlorophenyl)-2-furoyl]amino}butanoic acid (50e)

The title compound was obtained as an off-white solid in 100% yield from **49e** according to the analogous procedures as described for the preparation of **50a**: TLC $R_f = 0.20$ ($CHCl_3$–MeOH, 9:1); MS (MALDI, pos.) *m/z* 330 $(M+Na)^+$, 308 $(M+H)^+$; ^1H NMR (200 MHz, DMSO-d_6): δ 12.07 (s, 1H), 8.62–8.51 (m, 1H), 7.94 (d, $J = 8.4$ Hz, 2H), 7.54 (d, $J = 8.4$ Hz, 2H), 7.17–7.11 (m, 2H), 3.33–3.22 (m, 2H), 2.29 (t, $J = 7.2$ Hz, 2H), 1.77 (quintet, $J = 7.2$ Hz, 2H).

5.47. *trans*-*N*-(4-Hydroxyamino-4-oxobutyl)-4-methylcyclohexanecarboxamide (14)

To a stirred solution of **50a** (600 mg, 2.64 mmol) in DMF (3 mL) were added 1-hydroxybenzotriazole monohydrate (810 mg, 5.29 mmol), 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (1.01 g, 5.29 mmol), and *O*-(2-methoxy-2-propyl)hydroxylamine (555 mg, 5.29 mmol) at room temperature. After stirring at room temperature for 3 h, the reaction mixture was diluted with water and extracted with EtOAc. The organic layer was washed with saturated NH_4Cl, saturated $NaHCO_3$, and brine. The organic layer was dried over $MgSO_4$. Removal of the volatiles in vacuo provided an oily residue, which was dissolved in MeOH (3 mL). The resulting solution was acidified with 1 N HCl to pH 3. After stirring at room temperature for 30 min, the solution was concentrated in vacuo. The obtained residue was triturated with Et_2O to give **14** (217 mg, 34% yield) as a white powder: TLC $R_f = 0.29$ ($CHCl_3$–MeOH–AcOH, 18:2:1); MS (MALDI, pos.) *m/z* 281 $(M+K)^+$, 265 $(M+Na)^+$; IR (KBr) 3306, 2928, 1616, 1546, 1440, 1265, 1222, 1188, 1048, 1000, 949, 720, 661, 580, 544, 441 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6): δ 10.37 (s, 1H), 10.20 (s, 1H), 7.69 (t, $J = 5.3$ Hz, 1H), 3.07–2.92 (m, 2H), 2.31–1.88 (m, 3H), 1.74–1.52 (m, 6H), 1.46–1.18 (m, 3H), 0.98–0.76 (m, 2H), 0.85 (d, $J = 6.6$ Hz, 3H); HRMS (FAB) calcd for $C_{12}H_{23}N_2O_3$: 243.1709. Found: 243.1708.

5.48. *trans*-*N*-(4-Hydroxyamino-4-oxobuty)-4-propylcyclohexanecarboxamide (15)

The title compound was obtained as a white solid in 91% yield from **50b** according to the analogous procedures as described for the preparation of **14**: TLC $R_f = 0.34$ ($CHCl_3$–MeOH–AcOH, 18:2:1); MS (MALDI, pos.) *m/z* 309 $(M+K)^+$, 293 $(M+Na)^+$, 271 $(M+H)^+$; IR (KBr) 3283, 2927, 2852, 1651, 1561, 1444, 1392, 1266, 1208, 1048, 997, 948, 934, 717, 580, 534 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6): δ 10.35 (s, 1H), 7.67 (t, $J = 5.3$ Hz, 1H), 2.99 (q, $J = 6.5$ Hz, 2H), 2.39–1.88 (m, 3H), 1.78–1.61 (m, 6H), 1.45–1.07 (m, 7H), 0.95–0.76 (m, 5H); HRMS (FAB) calcd for $C_{14}H_{27}N_2O_3$: 271.2022. Found: 271.2026.

5.49. *N*-(4-Hydroxyamino-4-oxobutyl)-1-benzofuran-2-carboxamide (16)

The title compound was obtained as a white powder in 47% yield from **50c** according to the analogous procedures as described for the preparation of **14**: TLC $R_f = 0.16$ ($CHCl_3$–MeOH–AcOH, 100:10:1); MS (FAB, pos.) *m/z* 263 $(M+H)^+$; IR (KBr) 3306, 3218, 1644, 1603, 1547, 1448, 1419, 1305, 1260, 1183, 969, 826, 745, 722 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6): δ 10.38 (br s, 1H), 9.30–8.10 (br, 1H), 8.75 (t, $J = 6.2$Hz, 1H), 7.76 (m, 1H), 7.64 (m, 1H), 7.51 (d, $J = 0.6$ Hz, 1H), 7.45 (td, $J = 7.0$, 1.6 Hz, 1H), 7.32 (td, $J = 7.6$, 1.0 Hz, 1H), 3.25 (q, $J = 6.2$ Hz, 2H), 2.01 (t, $J = 7.0$ Hz, 2H), 1.74 (m, 2H); HRMS (FAB) calcd for $C_{13}H_{15}N_2O_4$: 263.1032. Found: 263.1033.

5.50. *N*-(4-Hydroxyamino-4-oxobutyl)-5-[(4-methylphenyl)ethynyl]-2-furamide (17)

The title compound was obtained as a yellow powder in 48% yield from **50d** according to the analogous procedures as described for the preparation of **14**: TLC $R_f = 0.32$ ($CHCl_3$–MeOH–AcOH, 18:2:1); MS (MALDI, pos.) *m/z* 349 $(M+Na)^+$, 327 $(M+H)^+$; IR (KBr)

3306, 2210, 1650, 1549, 1439, 1369, 1321, 1225, 1187, 1038, 968, 811, 759, 594, 523 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6): δ 10.40 (br s, 1H), 10.22 (s, 1H), 8.56 (t, J = 5.7 Hz, 1H), 7.47 (d, J = 8.3 Hz, 2H), 7.28 (d, J = 8.3 Hz, 2H), 7.16 (d, J = 3.6 Hz, 1H), 6.96 (d, J = 3.6 Hz, 1H), 3.28–3.14 (m, 2H), 2.36 (s, 3H), 2.00 (t, J = 7.5 Hz, 2H), 1.83–1.64 (m, 2H); HRMS (FAB) calcd for $C_{18}H_{19}N_2O_4$: 327.1345. Found: 327.1347.

5.51. 5-(4-Chlorophenyl)-*N*-(4-hydroxyamino-4-oxobutyl)-2-furamide (18)

The title compound was obtained as a white powder in 62% yield from **50e** according to the analogous procedures as described for the preparation of **14**: TLC R_f = 0.38 ($CHCl_3$–MeOH–AcOH, 18:2:1); MS (FAB, pos.) *m/z* 323 $(M+H)^+$; IR (KBr) 3205, 3034, 2874, 1739, 1624, 1573, 1546, 1512, 1476, 1303, 1273, 1215, 1187, 1170, 1093, 1043, 1019, 831, 799, 734, 663, 599 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6): δ 10.40 (s, 1H), 8.64–8.51 (m, 1H), 7.95 (d, J = 8.4 Hz, 2H), 7.54 (d, J = 8.4 Hz, 2H), 7.16–7.11 (m, 2H), 3.31–3.18 (m, 2H), 2.08–1.95 (m, 2H), 1.76 (quintet, J = 7.0 Hz, 2H); HRMS (FAB) calcd for $C_{15}H_{16}ClN_2O_4$: 323.0799. Found: 323.0797.

6. Biology. Enzyme assays: MMP-1, MMP-2, MMP-9, and MMP-3 assays using synthetic substrate

6.1. MMP-1 assay

Commercially available assay kits (Yagai, Yamagata City, Japan) were used. The solutions provided in the kits were used unless otherwise stated. A 98 μL portion of enzyme solution (0.5 unit/mL) and 2 μL of inhibitor solution (DMSO) were incubated with 100 μL of 0.5 mg/mL FITC-labeled type I collagen solution at 37 °C for 3 h. After incubation with 300 L of quenching solution on ice for 30 min, the reaction mixture was centrifuged at 2000*g* for 15 min. Supernatant was used for measurement of fluorescence by RF5300-PC. Excitation and emission wavelengths were 495 and 520 nm, respectively.

6.2. MMP-2 and MMP-9 assay[6]

A mixture of 130 μL of MOCAc-Pro-Leu-Gly-Leu-Dpa-Ala-Arg-NH_2 (final concentration: 15 μM) and 20 μL of inhibitor solution were incubated at 37 °C for 5 min. 50 μL of enzyme solution was added, and the reaction was performed at 37 °C for 10 min. Its fluorescence was measured by fmax. Excitation and emission wavelengths were 320 and 390 nm, respectively.

6.3. MMP-3 assay[7]

A mixture of 190 μL of MOCAc-Arg-Pro-Lys-Pro-Val-Glu-Nva-Trp-Arg-Lys(Dnp)-NH_2 (final concentration: 15 μM) and 10 μL of inhibitor solution were incubated at 37 °C for 5 min. 50 μL of enzyme solution was added and the reaction was performed at 37 °C for 10 min. Its fluorescence was measured by fmax. Excitation and emission wavelengths were 320 and 390 nm, respectively.

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