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Design and synthesis of novel metalloproteinase inhibitors

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Abstract—A series of *N*-benzoyl 4-aminobutyric acid hydroxamate analogs were synthesized and evaluated as matrix metalloproteinase inhibitors. Synthetic work was focused on the chemical modification of the 4-aminobutyric acid part using easily available starting materials. As such, chemical modification was carried out using commercially available starting materials such as 4-aminobutyric acid, (+)- and (−)-malic acid, and D- and L-glutamic acid derivatives. Among the compounds tested, *N*-[4-(benzofuran-2-yl)benzoyl] 4-amino-4*S*-hydroxymethylbutyric acid hydroxamates derived from L-glutamic acid demonstrated more potent inhibitory activity against MMP-2 and MMP-9 compared with the corresponding 2*S*-hydroxy analogs or 3*S*-hydroxy analogs, respectively, which were derived from (−)-malic acid. Structure–activity relationship study is presented.

1. Introduction

Matrix metalloproteinases (MMPs) are a family of zinc-containing endoproteinases that have been traditionally characterized by their collective ability to degrade all components of the extracellular matrix. These enzymes are postulated to regulate the homeostasis of a variety of tissues under the control of tissue inhibitor of metalloproteinases (TIMPs), which bind to and inhibit the activity of MMPs. Accordingly, an imbalance between MMPs and TIMPs can lead to a variety of pathological states, such as metastasis of cancer, or diseases including, rheumatoid arthritis and multiple sclerosis. At least 20 members of this enzyme family, which demonstrate significant sequence homology, have been reported.[1,2] They can be divided into collagenases (MMP-1, -8, -13, and -18), gelatinases (MMP-2 and -9), stromelysins (MMP-3, -10, and -11), and membrane-type MMPs (MMP-14, -15, -16, and -17). For more than 30 years MMPs have been seen as promising targets for the treatment of the above-mentioned diseases because collagenase (MMP-1), gelatinases (MMP-2 and -9), and stromelysin-I (MMP-3) have been shown to play a key role in cancer invasion and metastasis; angiogenesis and tumor metastasis; carcinogenesis and tumor growth, respectively.[3–5] Accordingly inhibitors of the gelatinases may have therapeutic potential for angiogenesis and/or tumor metastasis.

A large number of succinyl hydroxamates and sulfonamide hydroxamates have been reported as MMP inhibitors.[6] In our previous paper, we reported on the discovery of *N*-benzoyl 4-aminobutyric acid hydroxamate **1** (Table 1) as a new chemical lead for an MMP inhibitor (MMPI) by the fragment-based lead generation procedure.[7] Here, we report on the further optimization of **1** and on identification of novel MMPIs derived from malic acid and glutamic acid.

2. Chemistry

The compounds listed in Tables 1–5 were synthesized as described in Schemes 1–11. Compound **1** was prepared as described in Scheme 1. N-Benzoylation of 4-aminobutyric acid with *p*-toluoyl chloride **22** under alkaline condition afforded **23**. Condensation of **23** with a protected hydroxylamine hydrochloride led to **24**, catalytic hydrogenation of which provided the hydroxamate **1**.

Synthesis of **2** is outlined in Scheme 2. Suzuki coupling of **25** with benzofuran-2-boronic acid in the presence of a palladium catalyst produced **26**. Alkaline hydrolysis of

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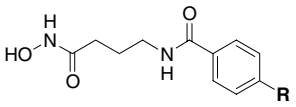
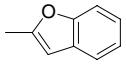
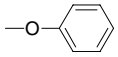
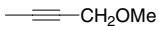
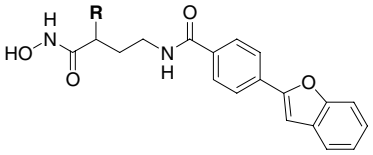
Table 1. Effect of the *p*-substituent of the *N*-benzoyl residue on the activity profiles

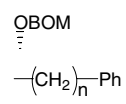


Compound	R	IC_{50}[a,d] (μM)			
		MMP-1	MMP-2	MMP-9	MMP-3
1	–Me	59%[b]	18	>100	NT[c]
2		53%[b]	0.0029	0.054	1.8
3		>100	0.038	0.012	2.9
4		9.9	0.047	NT	16

[a] Concentration required for 50% inhibition of enzyme activity.
[b] Inhibition percentage at 100 μM.
[c] Not tested.
[d] IC_{50} values were determined in a single experimental run in duplicate.

Table 2. Effect of the C2-substitution on the activity profiles



Compound	R	IC_{50}[a,c] (μM)			
		MMP-1	MMP-2	MMP-9	MMP-3
2	H	53%[b]	0.0029	0.054	1.8
5		56%[b]	0.0033	0.053	1.3
6		44%[b]	0.0069	NT	8.2
7		46%[b]	0.0036	0.024	0.74
8		35	0.78	NT	44%[b]
9	$n = 1$	32	0.035	0.057	0.16
10	$n = 2$	>100	0.021	NT	0.83
11	$n = 3$	36%[b]	0.017	0.043	0.25

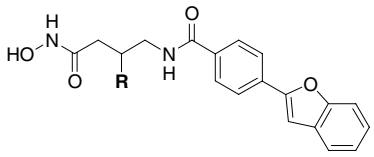
BOM, benzyloxymethyl.
[a] Concentration required for 50% inhibition of enzyme activity.
[b] Inhibition percentage at 100 μM.
[c] IC_{50} values were determined in a single experimental run in duplicate.

the ester **26** resulted in **27**. Condensation of the carboxylic acid **27** with 4-aminobutyric acid ethyl ester in the presence of EDC afforded **28**. Alkaline hydrolysis of **28** produced **29**, condensation of which with a protected hydroxylamine in the presence of EDC, followed by acidic deprotection, gave compound **2**.

Compound **3** was prepared as described in Scheme 3. Condensation of a carboxylic acid **30** with 4-aminobutyric acid ethyl ester in the presence of EDC afforded **31**, alkaline hydrolysis of which produced the corresponding carboxylic acid **32**. Condensation of **32** with a protected hydroxylamine in the presence of EDC, followed by deprotection, led to compound **3**.

Compound **4** was prepared from **35**, which was obtained by palladium-catalyzed coupling reaction of methyl 4-iodobenzoate **33** with 3-methoxy-1-propyne, followed by alkaline hydrolysis, as described in Scheme 4. Condensation of **35** with 4-aminobutyric acid ethyl ester produced **36**, alkaline hydrolysis of which provided **37**. Condensation of **37** with a protected hydroxylamine, followed by acidic deprotection, afforded compound **4**.

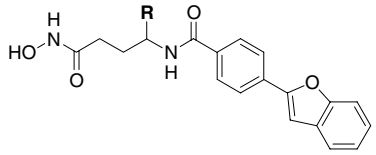
Table 3. Effect of the C3-substitution on the activity profiles



Compound	R	IC_{50}[a,c] (μM)			
		MMP-1	MMP-2	MMP-9	MMP-3
2	H	53%[b]	0.0029	0.054	1.8
12	OH	38%[b]	0.0050	0.093	1.5
13	OH	>100	0.026	NT	10
14	OMOM	46%[b]	0.025	0.16	1.5
15	OMOM	>100	0.026	NT	7.7

MOM, methoxymethyl.
[a] Concentration required for 50% inhibition of enzyme activity.
[b] Inhibition percentage at 100 μM.
[c] IC_{50} values were determined in a single experimental run in duplicate.

Table 4. Effect of the C4-substitution on the activity profiles

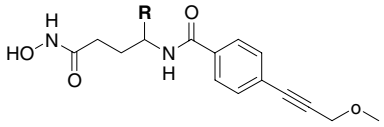


Compound	R	IC_{50}[a,c] (μM)			
		MMP-1	MMP-2	MMP-9	MMP-3
2	H	53%[b]	0.0029	0.054	1.8
16	OH	2.6	0.0019	0.012	0.54
17	OMOM	1.5	0.00073	0.0077	0.23

MOM, methoxymethyl.
[a] Concentration required for 50% inhibition of enzyme activity.
[b] Inhibition percentage at 100 μM.
[c] IC_{50} values were determined in a single experimental run in duplicate.

Synthesis of optically active analogs **5**–**8** from the malic acids possessing the corresponding absolute configurations is outlined in Scheme 5. Reduction of the carboxylic acid of **38a**,**b** with diborane produced alcohols **39a**,**b**, respectively, tosylation of which resulted in **40a**,**b**, respectively. The tosylates **40a**,**b** were substituted by sodium azide to produce **41a**,**b**, respectively. Acidic deprotection of **41a**,**b** in the presence of methanol led to **42a**,**b**, respectively. Catalytic hydrogenation of **42a**,**b**, followed by N-acylation with an acid chloride **43** prepared from the corresponding carboxylic acid, gave **44a**,**b**, respectively. Alkaline hydrolysis of **44a**,**b** gave **45a**,**b**, respectively, which were converted to the corresponding hydroxamates **5** and **6**, respectively, in the usual manner. O-Alkylation of **44a**,**b** with a benzyloxymethylchloride (BOMCl) resulted in the formation of **46a**,**b**, respectively. Alkaline hydrolysis of **46a**,**b** gave the corresponding carboxylic acids **47a**,**b**, respectively,

Table 5. Effect of the C4-substitution of another *N*-benzoyl analogs on the activity profiles



Compound	R	IC_{50}[a,b] (μM)			
		MMP-1	MMP-2	MMP-9	MMP-3
4	-H	9.9	0.047	NT	16
18	OH	2.1	0.022	NT	4.7
19	OH	4.5	0.049	NT	11
20	OMOM	1.1	0.0088	NT	2.0
21	OMOM	1.7	0.016	NT	3.1

MOM, methoxymethyl.
[a] Concentration required for 50% inhibition of enzyme activity.
[b] IC_{50} values were determined in a single experimental run in duplicate.

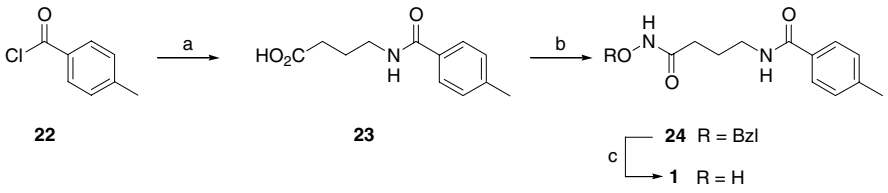
which were converted to their corresponding hydroxamates **7** and **8**, respectively, by the usual procedures.

Synthesis of **9** from **49**, which was prepared by N-benzoylation of 4-aminobutyric acid ethyl ester, is outlined in Scheme 6. C2-Alkylation of **49** under conventional reaction conditions yielded **50**, which was converted to **51** by the palladium-catalyzed coupling reaction with benzofuran-2-boronic acid. Alkaline hydrolysis of **51** provided **52**, which was converted to the hydroxamate **9** by the usual procedure.

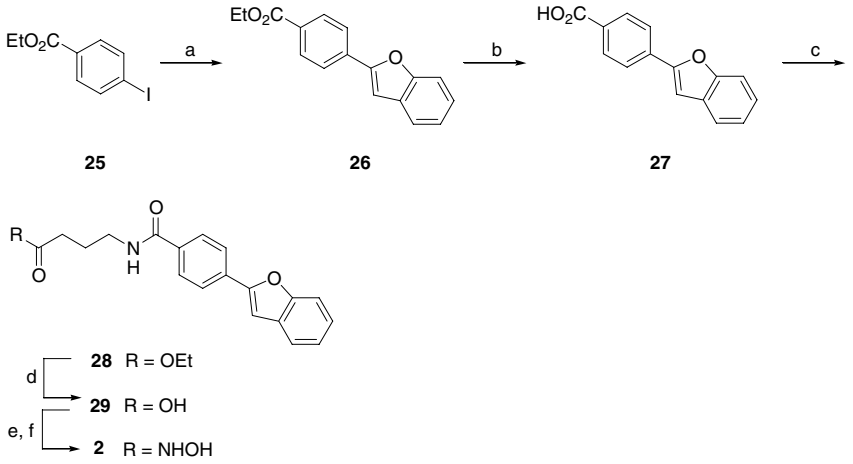
Synthesis of **10** is outlined in Scheme 7. Alkylation of **53** with bromoacetonitrile resulted in **54**, which was reduced to an amino ester **55**. N-Acylation of **55** with the acid chloride **43** produced **56**, alkaline hydrolysis of which afforded **57**. Compound **57** was converted to the corresponding hydroxamate **10** in the usual manner.

Synthesis of **11** is described in Scheme 8. C2-Alkylation of **49** with 1-bromo-3-phenyl-2-propene gave **58**. Palladium-catalyzed Suzuki coupling of **58** with the benzofuran-2-yl boronic acid afforded **59**, and catalytic hydrogenation of **59** produced **60**. Then compound **60** was converted to the hydroxamate **11** according to the procedure described above.

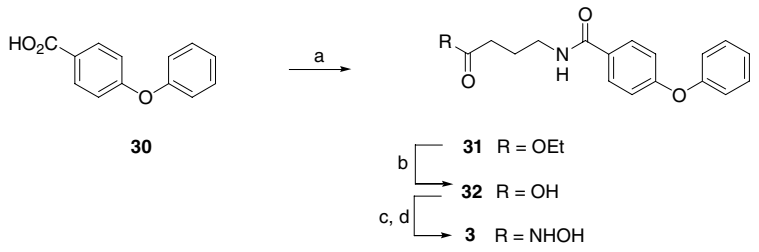
Synthesis of **12**–**15** is outlined in Scheme 9. The selective reduction of optically active malic acid methyl esters **62a**,**b** yielded **63a**,**b**, respectively.[8] Tosylation of **63a**,**b** afforded **64a**,**b**, respectively, substitution of which with sodium azide produced **65a**,**b**, respectively. Catalytic hydrogenation of the azides **65a**,**b**, followed by N-acylation of the amino function thus formed, led to **66a**,**b**, respectively. Alkaline hydrolysis of **66a**,**b**, followed by formation of the hydroxamates **12** and **13**, was carried out according to the usual procedures, as described above. O-Alkylation of **66a**,**b** with methoxymethyl chloride (MOMCl) provided **68a**,**b**, respectively, while alkaline hydrolysis of **68a**,**b** afforded **69a**,**b**, respectively.



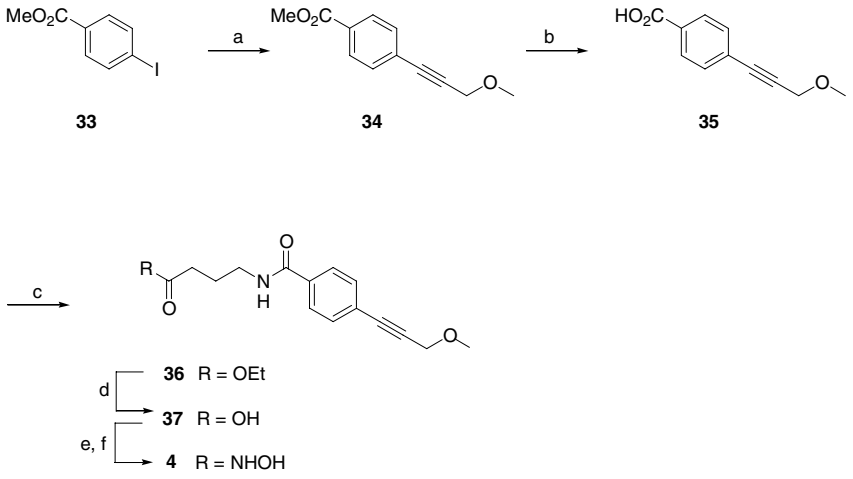
Scheme 1. Synthesis of **1**. Reagents: (a) 4-aminobutyric acid, NaOH aq, THF; (b) $HCl{\cdot}NH_2OBzl$, EDC, HOBt, $^iPr_2NEt$, DMF; (c) H_2, Pd–C, MeOH.



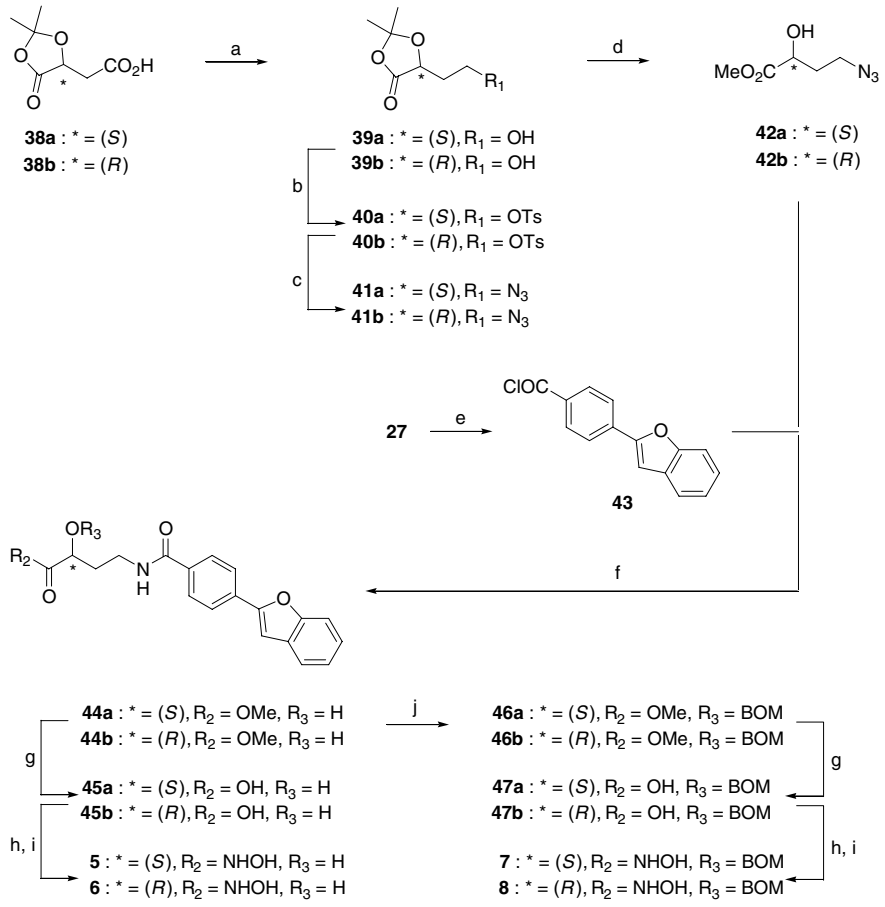
Scheme 2. Synthesis of **2**. Reagents: (a) benzofuran-2-boronic acid, $PdCl_2(PPh_3)_2$, Et_3N, DMF; (b) NaOH aq, dioxane; (c) 4-aminobutyric acid ethyl ester hydrochloride, EDC, HOBt, Et_3N, DMF; (d) NaOH aq, THF; (e) *O*-(2-methoxypropane-2-yl)hydroxylamine, EDC, HOBt, DMF; (f) HCl, MeOH.



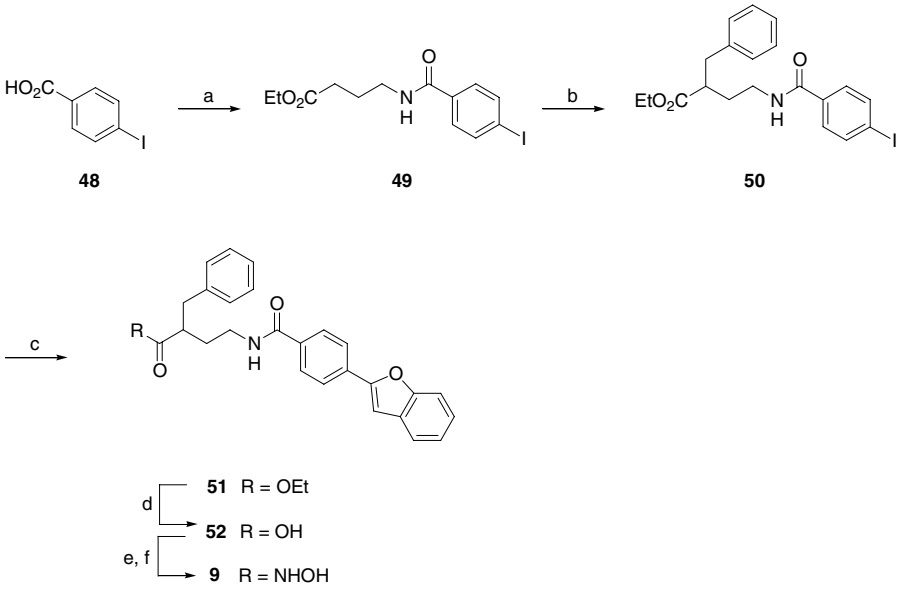
Scheme 3. Synthesis of **3**. Reagents: (a) 4-aminobutyric acid ethyl ester hydrochloride, EDC, HOBt, Et_3N, DMF; (b) NaOH aq, THF; (c) *O*-(2-methoxypropane-2-yl)hydroxylamine, EDC, HOBt, DMF; (d) HCl, MeOH.



Scheme 4. Synthesis of **4**. Reagents: (a) 3-methoxy-1-propyne, $PdCl_2(PPh_3)_2$, Et_3N, DMF; (b) NaOH aq, DMF–MeOH; (c) 4-aminobutyric acid ethyl ester hydrochloride, EDC, HOBt, Et_3N, DMF; (d) NaOH aq, THF; (e) *O*-(2-methoxypropane-2-yl)hydroxylamine, EDC, HOBt, DMF; (f) HCl, MeOH.



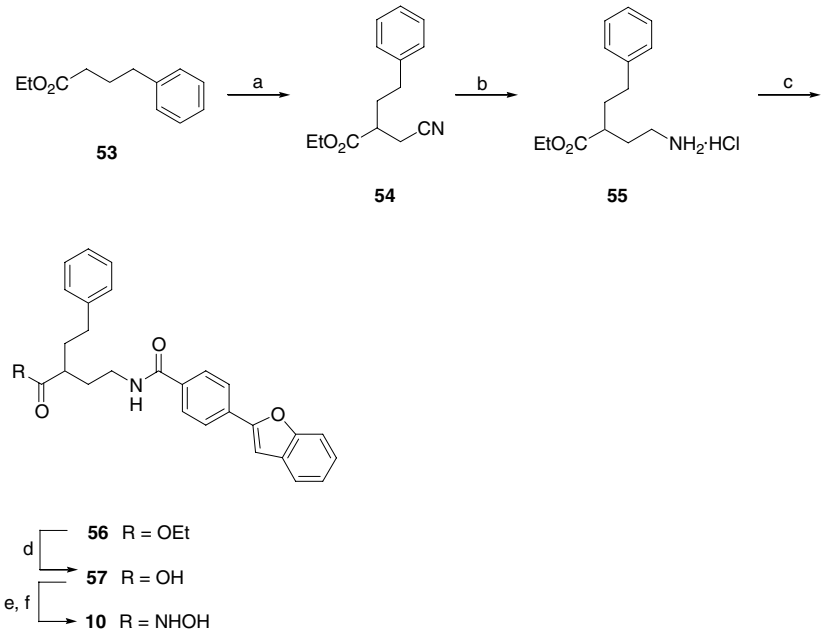
Scheme 5. Synthesis of **5**–**8**. Reagents: (a) BH_3–THF, THF; (b) TsCl, pyridine; (c) NaN_3, DMF; (d) TsOH, MeOH; (e) $SOCl_2$; (f) i—H_2, Pd–C, 4 N HCl/MeOH, MeOH; ii—Et_3N, CH_2Cl_2; (g) NaOH aq, THF–MeOH; (h) *O*-(2-methoxypropane-2-yl)hydroxylamine, EDC, HOBt, DMF; (i) HCl, MeOH; (j) BOMCl, $^iPr_2NEt$, CH_2Cl_2.



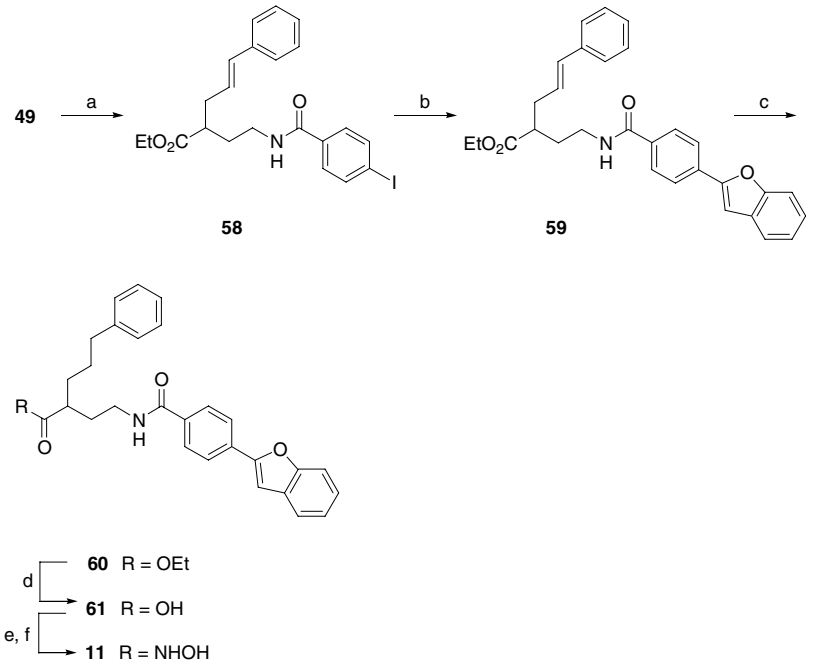
Scheme 6. Synthesis of **9**. Reagents: (a) 4-aminobutyric acid ethyl ester hydrochloride, EDC, HOBt, Et_3N, DMF; (b) LiHMDS, BnBr, THF; (c) benzofuran-2-boronic acid, $PdCl_2(PPh_3)_2$, Et_3N, DMF; (d) NaOH aq, THF–MeOH; (e) *O*-(2-methoxypropane-2-yl)hydroxylamine, EDC, HOBt, DMF; (f) HCl, MeOH.

Formation of the hydroxamates **14** and **15** was carried out according to the usual procedures, as described above.

Synthesis of **16** and **17** is described in Scheme 10. Compound **71**, which was prepared by N-acylation of γ-methyl L-glutamate **70** with **43**, was converted to **72** by



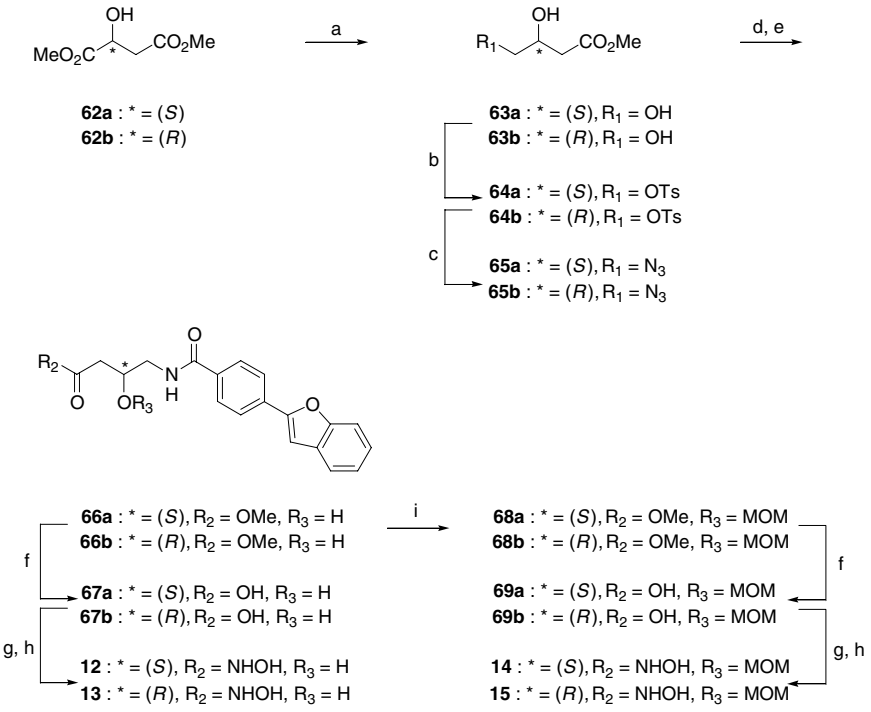
Scheme 7. Synthesis of **10**. Reagents: (a) LDA, bromoacetonitrile, THF; (b) H_2, Pt_2O, 4 N HCl/EtOAc, MeOH; (c) **43**, Et_3N, CH_2Cl_2; (d) NaOH aq, THF; (e) *O*-(2-methoxypropane-2-yl)hydroxylamine, EDC, HOBt, DMF; (f) HCl, MeOH.



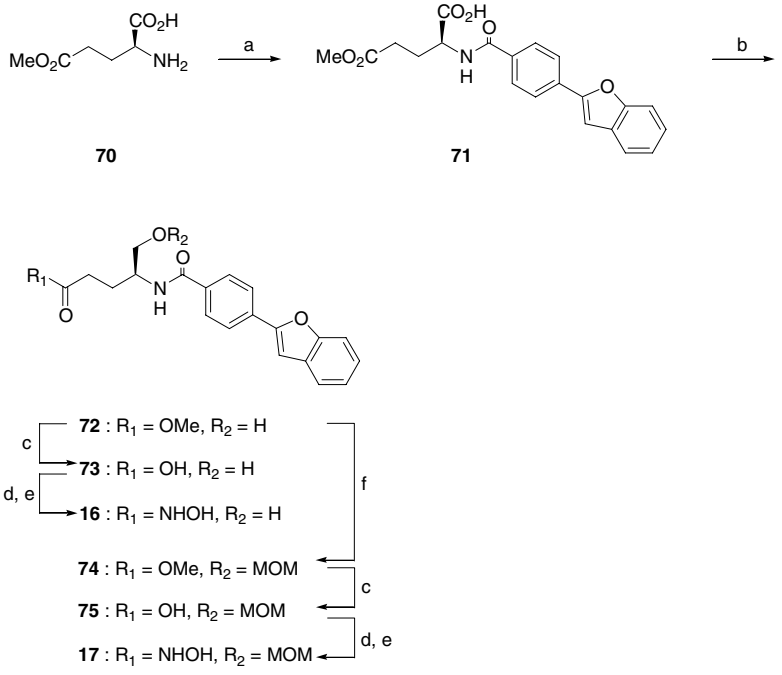
Scheme 8. Synthesis of **11**. Reagents: (a) LiHMDS, 1-bromo-3-phenyl-2-propene, THF; (b) benzofuran-2-boronic acid, $PdCl_2(PPh_3)_2$, Et_3N, DMF; (c) H_2, Pd–C, MeOH–DMF; (d) NaOH aq, THF–MeOH; (e) *O*-(2-methoxypropane-2-yl)hydroxylamine, EDC, HOBt, DMF; (f) HCl, MeOH.

hydroboration with diborane. Alkaline hydrolysis of **72**, followed by formation of the hydroxamate, was carried out according to the usual procedures to produce **16**. O-Alkylation of **72** with MOMCl gave **74**, alkaline hydrolysis of which afforded **75**. The hydroxamate **17** was obtained from the carboxylic acid **75** in the usual manner.

Synthesis of optically active analogs **18**–**21** is outlined in Scheme 11. N-Acylation of optically active glutamic acid γ-methyl esters **70** and **76** with 4-iodobenzoyl chloride provided **77a**,**b**, respectively. Hydride reduction of the corresponding activated esters of **77a**,**b** gave **78a**,**b**, respectively. Palladium-catalyzed coupling reaction of **78a**,**b** with an alkyne provided **79a**,**b**, respectively. Alkaline hydrolysis of **79a**,**b**, followed by hydroxamate formation by the usual procedures, resulted in **18** and **19**, respectively. O-Alkylation of **79a**,**b** with MOMCl provided **81a**,**b**, respectively. Alkaline hydrolysis of **81a**,**b**, followed by hydroxamate formation by the usual procedures, led to **20** and **21**, respectively.



Scheme 9. Synthesis of **12**–**15**. Reagents: (a) BH_3–SMe_2, $NaBH_4$, THF; (b) TsCl, pyridine; (c) NaN_3, DMF; (d) H_2, Pd–C, 4 N HCl/MeOH, MeOH; (e) **43**, Et_3N, CH_2Cl_2; (f) NaOH aq, THF–MeOH; (g) *O*-(2-methoxypropane-2-yl)hydroxylamine, EDC, HOBt, DMF; (h) HCl, MeOH; (i) MOMCl, $^iPr_2NEt$, CH_2Cl_2.

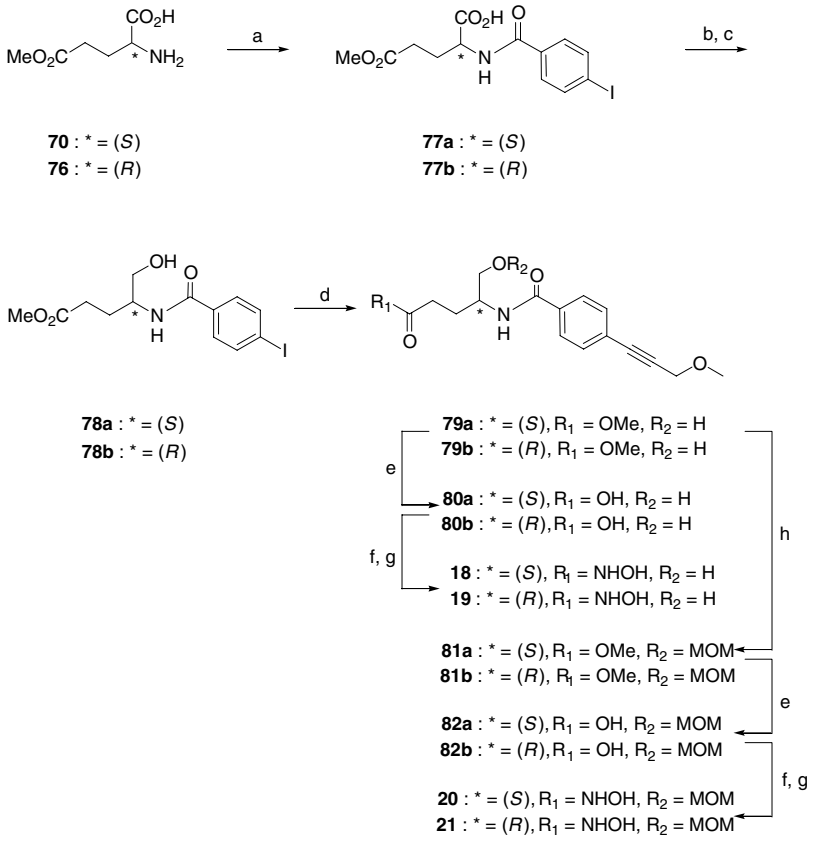


Scheme 10. Synthesis of **16** and **17**. Reagents : (a) **43**, $NaHCO_3$, THF–H_2O; (b) BH_3–THF, THF; (c) NaOH aq, MeOH; (d) *O*-(2-methoxypropane-2-yl)hydroxylamine, EDC, HOBt, DMF; (e) HCl, MeOH; (f) MOMCl, $^iPr_2NEt$, CH_2Cl_2–THF.

3. Results and discussion

The compounds listed in Tables 1–5 were tested to assess their inhibitory potency against MMP-1 (human collagenase), MMP-2 (human gelatinase A), MMP-9 (human gelatinase B), and MMP-3 (recombinant human stromelysin).[9] Since the discovery of *N*-benzoyl 4-aminobutyric acid hydroxamate as a new chemical lead for an inhibitor of MMP-2 and MMP-9, further chemical modification to identify the optimum structure has been continued.

As described in our previous paper,[7] C2 substitution of the 4-aminobutyric acid moiety tended to show an



Scheme 11. Synthesis of **18**–**21**. Reagents: (a) 4-iodobenzoyl chloride, $NaHCO_3$, Et_2O–H_2O; (b) HONSu, DCC, THF; (c) $NaBH_4$, THF–H_2O; (d) 3-methoxy-1-propyne, $Pd(PPh_3)_4$, CuI, Et_3N, DMF or 3-methoxy-1-propyne, $PdCl_2(PPh_3)_2$, Et_3N, DMF; (e) NaOH aq, THF–MeOH; (f) *O*-(2-methoxypropane-2-yl)hydroxylamine, EDC, HOBt, DMF; (g) HCl, MeOH; (h) MOMCl, $^iPr_2NEt$, CH_2Cl_2.

increased inhibitory potency and chemical modification of C1 carboxylic acid residue of the glutamic acid moiety was useful to improve subtype selectivity and/or inhibitory potency. We also reported on the optimization process of the *N*-benzoyl moiety of **1** as described in Table 1.[10] Replacement of the *para*-methyl residue of the *N*-benzoyl moiety of **1** with a benzofuran-2-yl, phenoxy and methoxymethyl-1-propyn-1-yl residues afforded **2**–**4**, respectively, with an increased inhibitory potency especially against MMP-2 and MMP-9 isoforms. On the basis of the above-mentioned results, further chemical modification of the 4-aminobutyric acid moiety of *N*-(4-benzofuran-2-yl) benzoyl-4-aminobutyric acid hydroxamate **2** was conducted as shown in Tables 2–4.

For the efficient synthetic work, several commercially available starting materials such as 4-aminobutyric acid, (+)- and (−)-malic acid, and D- and L-glutamic acid were selected. Using these starting materials, chemical modifications producing three series of scaffolds A–C (Fig. 1) could be developed.

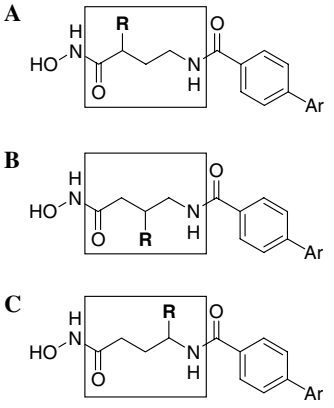


Figure 1. Three scaffolds A–C derived from commercially available starting materials.

As shown in Table 2, synthesis and evaluation was carried out for 2-hydroxy analogs of the 4-aminobutyric acid, which was synthesized as an optically active compound from the corresponding malic acid. The 2*S*-hydroxy analog **5** showed similar inhibitory activity for MMP-1, MMP-2, MMP-9, and MMP-3 to that of **2**, while 2*R*-analog **6** was a nearly 2-fold less potent inhibitor of MMP-2 and was a 4.5-fold less potent inhibitor of MMP-3. The 2*S*-benzyloxymethyloxy analog **7** was an equipotent and 2.2-fold more potent inhibitor of MMP-2 and MMP-9 relative to **2**, respectively, while it retained the same activity and showed 2.4-fold more potent activity for MMP-1 and MMP-3, respectively. The 2*R*-benzyloxymethyloxy analog **8** showed a marked reduction of inhibitory activity, especially for MMP-2, because the introduction of a bulky substituent led to undesirable conformation.

Based on the results for **7**, synthesis and evaluation of the 2-phenylalkyl analogs **9**–**11** was carried out as described in Table 2. These compounds were synthesized and evaluated as a racemic mixture of 2*R*- and 2*S*-forms.

Introduction of a benzyl moiety into the C2-position of **2** afforded **9**, with 12-fold less potent inhibitory activity, equipotent activity, and nearly 10-fold more potent activity for MMP-2, MMP-9, and MMP-3, respectively. Introduction of phenylethyl and phenylpropyl moieties instead of a benzyl moiety at the C2-position of **2** gave **10** and **11**, respectively, which also showed less potent inhibition of MMP-2, while **11** retained its activity against MMP-9. Interestingly, these three compounds (**9**–**11**) all showed to be more potent inhibitors of MMP-3 relative to **2**. Thus, these C2-modifications failed to significantly increase the inhibitory activity for MMP-2 and MMP-9, while some of them showed slightly increased inhibition of MMP-3, as illustrated by compounds **7** and **9**–**10**.

Chemical modification of C3 on compound **2** was also carried out, as shown in Table 3. Again, the synthesis and evaluation of 3-hydroxy analogs derived from malic acid was conducted due to the ease of synthesis. Introduction of a 3*S*-hydroxy moiety into **2** afforded **12**, which showed slightly weaker inhibitory activities for MMP-2 and MMP-9. The corresponding 3*R*-hydroxy isomer **13** demonstrated nearly 10-fold less potent inhibitory activity for MMP-2 relative to that of **2**, while it showed 5.5-fold weaker inhibitory activity against MMP-3. Thus, the 3*S*-hydroxy analog **12** showed 5.2-fold more potent inhibitory activity for MMP-2 than the corresponding 3*R*-hydroxy analog. The corresponding derivatives **14** and **15** were also evaluated. Both the 3*S*-methoxymethyloxy analog **14** and the corresponding 3*R*-isomer **15** showed 8.6-fold less potent inhibitory activity for MMP-2 regardless of their stereochemistry. Thus, the pocket for the C3-substituent seemed to be smaller than that accepting the C2-substituent.

As shown in Table 4, chemical modification of the C4 position of compound **2** was carried out with the 4*S*-isomer, because it was predicted to be more potent than the corresponding 4*R*-isomer. Synthesis and evaluation of 4*S*-hydroxymethyl analogs, which could be derived from L-glutamic acid, was also carried out due to the ease of synthesis. Introduction of a 4*S*-hydroxymethyl residue into **2** afforded **16**, which showed an equipotent, 4.5-fold more potent, and 3.3-fold more potent inhibition of MMP-2, MMP-9, and MMP-3, respectively. Introduction of the 4*S*-methoxymethyloxy residue into **2** resulted in **17**, which showed nearly 4.0-, 7.0-, and 7.8-folds more potent inhibitory activity for MMP-2, MMP-9, and MMP-3, respectively. As a result, chemical modification of C4 seemed to be most promising for further optimization of this series of analogs based on both the SAR data and the relative ease of synthesis.

As shown in Table 5, further chemical modification of the *para*-substituent of the *N*-benzoyl residue was continued using the most optimum partial structure, that is, the C4-substituted 4-aminobutyric acid. The 3-methoxymethyl-1-propyn-1-yl was selected as the *p*-substituent because it was included in the relatively optimum *N*-benzoyl residue **4** as a *p*-substituent. 4*S*-Hydroxymethyl analog **18** exhibited slightly more potent inhibition of MMP-2, while the corresponding 4*R*-hydroxymethyl analog **19** had nearly the same IC_{50} value as **4**. 4*S*-Methoxymethyloxy analog **20** showed 5.3-fold more potent inhibition of MMP-2 relative to **4**, while the corresponding 4*R*-isomer **21** showed 2.9-fold more potent inhibitory activity for MMP-2. Thus, all of the compounds listed in Table 5 showed less potent inhibition of MMP-2 than **16** and **17** in Table 4. As a result, optimization of the C4-substituent seemed to have priority over that of the *p*-substituent of the *N*-benzoyl residue.

In summary, chemical modification of the 4-aminobutyric acid moiety of **2** and **4** possessing the optimized *N*-benzoyl moieties was conducted using commercially available starting materials. Most of the compounds tested were highly potent inhibitors of the gelatinases (MMP-2 and MMP-9). Among the tested compounds, **16** and **17**, which were derived from L-glutamic acid, tended to show equipotent or slightly more potent inhibitory activities relative to **2** against all the isoforms. Further optimization works including in vivo and PK studies will be reported in due course. Compounds **9**, **11**, **16**, and **17** were also expected to be a promising chemical lead to identify a new MMP-3 inhibitor.

4. 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 parts per million (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; DMF, *N*,*N*-dimethylformamide; CH_2Cl_2, dichloromethane; $CHCl_3$, chloroform; EDC·HCl, 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride; HOBt, 1-hydroxybenzotriazole; Et_2O, diethyl ether; LiHMDS, lithium hexamethyldisilazide; LDA, lithium diisopropylamide; FITC, fluorescein isothiocyanate; MOCAc, 7-methoxycoumarin-4-acetyl; Dpa, *N*-3-(2,4-dinitrophenyl)-L-2,3-diaminopropionyl; Dnp, 2,4-dinitrophenyl.

4.1. 4-[(4-Methylbenzoyl)amino]butanoic acid (23)

To a stirred suspension of 4-aminobutyric acid (1.13 g, 11.0 mmol) in THF (21 mL) were added 1 N NaOH (21.0 mL, 21.0 mmol) at 0 °C and then **22** (1.55 g, 10.0 mmol). After being stirred 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 Et_2O to give 2.00 g (90%) of **23** as a white crystal: TLC $R_f = 0.58$ ($CHCl_3$–MeOH–AcOH, 18:2:1); ^1H 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).

4.2. *N*-[4-(Benzyloxy)amino-4-oxobutyl]-4-methylbenzamide (24)

To a stirred solution of **23** (885 mg, 4.00 mmol) in DMF (5 mL) were added *O*-benzylhydroxylamine hydrochloride (1.28 g, 8.00 mmol), *N*,*N*-diisopropylethylamine (3.48 mL, 20.0 mmol), HOBt·H_2O (1.35 g, 8.80 mmol), and EDC·HCl (1.69 g, 8.80 mmol). The reaction mixture was stirred at room temperature for 50 h, then diluted with EtOAc and washed sequentially 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 washed with Et_2O to give 986 mg (76%) of **24** as a white powder: TLC $R_f = 0.52$ ($CHCl_3$–MeOH, 10:1); ^1H 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).

4.3. *N*-[4-(Hydroxyamino)-4-oxobutyl]-4-methylbenzamide (1)

Catalytic hydrogenation of **24** (504 mg, 1.54 mmol) in MeOH (4 mL) was conducted at room temperature for 2 h in the presence of 10% palladium on carbon (50 mg) at atmospheric pressure. Removal of the catalyst by filtration, followed by evaporation, afforded a gray solid, which was purified by silica gel chromatography with MeOH–$CHCl_3$, 1:10, as an eluent. Evaporation of the product-rich fractions provided a solid, which was recrystallized from Et_2O to provide 311 mg (85%) of **1** as a white crystal: 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}; ^1H NMR (200 MHz, DMSO-d_6) δ 10.39 (1H, s), 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 (dt, $J = 6.6$, 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.

4.4. Ethyl 4-(1-benzofuran-2-yl)benzoate (26)

To a stirred solution of ethyl 4-iodobenzoate (**25**) (5.00 g, 18.1 mmol) in DMF (10 mL) were added benzofuran-2-boronic acid (2.64 g, 18.1 mmol), dichlorobis(triphenylphosphine)palladium(II) (635 mg, 0.905 mmol), and triethylamine (5 mL). After being stirred at 80 °C for 6 h, the reaction mixture was poured into 1 N HCl and extracted with EtOAc. The organic layer was washed with brine and dried over Na_2SO_4. Removal of the volatiles in vacuo provided a solid, which was washed with Et_2O–*n*-hexane to give 3.60 g (75%) of **26** as a light brown powder: TLC $R_f = 0.61$ (EtOAc–*n*-hexane, 1:9); ^1H NMR (300 MHz, $CDCl_3$) δ 8.12 (d, $J = 8.4$ Hz, 2H), 7.93 (d, $J = 8.4$ Hz, 2H), 7.65–7.58 (m, 1H), 7.58–7.51 (m, 1H), 7.37–7.21 (m, 2H), 7.16 (s, 1H), 4.41 (q, $J = 7.2$ Hz, 2H), 1.42 (t, $J = 7.2$ Hz, 3H).

4.5. 4-(1-Benzofuran-2-yl)benzoic acid (27)

To a stirred solution of **26** (3.40 g, 12.8 mmol) in dioxane (15 mL) was added 1 N NaOH (15.3 mL, 15.3 mmol). After being stirred at room temperature for 9 h, the reaction mixture was poured into 1 N HCl and extracted with EtOAc–THF. The organic layer was washed with brine and dried over $MgSO_4$. Removal of the volatiles in vacuo provided a crude solid, which was washed with EtOAc–Et_2O to give 2.10 g (69%) of **27** as a pale gray powder: TLC $R_f = 0.43$ ($CHCl_3$–MeOH–AcOH, 100:10:1); ^1H NMR (200 MHz, DMSO-d_6) δ 8.04 (m, 4H), 7.72–7.63 (m, 2H), 7.60 (br s, 1H), 7.41–7.24 (m, 2H).

4.6. Ethyl 4-{[4-(1-benzofuran-2-yl)benzoyl]amino}butanoate (28)

To a stirred solution of **27** (500 mg, 3.00 mmol) in DMF (15 mL) were added 4-aminobutyric acid ethyl ester hydrochloride (715 mg, 3.00 mmol), HOBt·H_2O (551 mg, 3.60 mmol), triethylamine (920 μL, 6.60 mmol), and EDC·HCl (690 mg, 3.60 mmol). After being stirred at room temperature for 2 days, the reaction mixture was poured into 1 N HCl and extracted with EtOAc–THF. The organic layer was washed with saturated $NaHCO_3$, brine and dried over Na_2SO_4. Removal of the volatiles in vacuo provided a solid, which was washed with EtOAc–Et_2O to give 732 mg (70%) of **28** as a white powder: TLC $R_f = 0.38$ (EtOAc–*n*-hexane, 1:1); ^1H NMR (200 MHz, DMSO-d_6) δ 8.57 (t, $J = 5.4$ Hz, 1H), 8.01 (d, $J = 9.2$ Hz, 2H), 7.96 (d, $J = 9.2$ Hz, 2H), 7.71–7.62 (m, 2H), 7.57 (br s, 1H), 7.39–7.23 (m, 2H), 4.04 (q, $J = 7.0$ Hz, 2H), 3.37–3.25 (m, 2H), 2.36 (t, $J = 7.4$ Hz, 2H), 1.86–1.72 (m 2H).

4.7. 4-{[4-(1-Benzofuran-2-yl)benzoyl]amino}butanoic acid (29)

To a stirred solution of **28** (670 mg, 1.91 mmol) in THF (5 mL) was added 1 N NaOH (4.40 mL, 4.40 mmol). After being stirred at room temperature for 3 h, the reaction mixture was poured into 1 N HCl and extracted with EtOAc–THF. The organic layer was washed with brine and dried over Na_2SO_4. Removal of the volatiles in vacuo provided 617 mg (100%) of **29** as a white powder, which was used for the next reaction without further purification: TLC $R_f = 0.40$ ($CHCl_3$–MeOH–AcOH, 100:10:1); ^1H NMR (200 MHz, DMSO-d_6) δ

8.58 (t, J = 5.6 Hz, 1H), 8.01 (d, J = 9.2 Hz, 2H), 7.96 (d, J = 9.2 Hz, 2H), 7.71–7.63 (m, 2H), 7.57 (d, J = 0.8 Hz, 1H), 7.39–7.23 (m, 2H), 3.32–3.25 (m, 2H), 2.29 (t, J = 7.6 Hz, 2H), 1.84–1.70 (m, 2H).

4.8. 4-(1-Benzofuran-2-yl)-*N*-[4-(hydroxyamino)-4-oxobutyl]benzamide (2)

To a stirred solution of **29** (550 mg, 1.70 mmol) in DMF (10 mL) at 0 °C were added *O*-(2-methoxypropane-2-yl)hydroxylamine (455 mg, 5.10 mmol), HOBt·H_2O (391 mg, 2.55 mmol), and EDC·HCl (489 mg, 2.55 mmol). After being stirred at room temperature for 3 h, the reaction mixture was poured into water and extracted with EtOAc. The organic layer was washed with saturated $NaHCO_3$, brine, and dried over Na_2SO_4. Removal of the volatiles in vacuo provided an oily residue, which was dissolved in MeOH (10 mL) and the resulting solution was acidified up to pH 3 with 1 N HCl. After being stirred at room temperature for 10 min, the reaction mixture was poured into water and extracted with EtOAc. The organic layer was washed with brine and dried over Na_2SO_4. Removal of the volatiles in vacuo provided a crude solid, which was washed with Et_2O to give 218 mg (38%) of **2** as a white powder: TLC R_f = 0.22 ($CHCl_3$–MeOH–AcOH, 100:10:1); MS (FAB, pos.) m/z 339 $(M+H)^+$; IR (KBr) 1633, 1541, 1451, 1306, 1030 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6): δ 10.39 (br s, 1H), 8.59 (t, J = 5.8 Hz, 1H), 8.01 (d, J = 9.0 Hz, 2H), 7.96 (d, J = 9.0 Hz, 2H), 7.67 (m, 2H), 7.57 (d, J = 0.5 Hz, 1H), 7.39–7.23 (m, 2H), 3.27 (q, J = 5.8 Hz, 2H), 2.03 (t, J = 7.6 Hz, 2H), 1.76 (m, 2H); HRMS (FAB) calcd for $C_{19}H_{19}N_2O_4$: 339.1345. Found: 339.1349.

4.9. Ethyl 4-[(4-phenoxybenzoyl)amino]butanoate (31) and 4-[(4-phenoxybenzoyl)amino]butanoic acid (32)

The ester **31** was obtained as a white powder in 75% yield from **30** according to the same procedures as described for the preparation of **28**. Compound **31**: TLC R_f = 0.76 ($CHCl_3$–MeOH–H_2O–AcOH, 100:10:1:1). The carboxylic acid **32** was obtained as a white powder in 98% yield from **31** according to the same procedures as described for the preparation of **29**. Compound **32**: TLC R_f = 0.47 ($CHCl_3$–MeOH–H_2O–AcOH, 100:10:1:1); ^1H NMR (200 MHz, DMSO-d_6) δ 8.41(t, J = 5.4 Hz, 1H), 7.85 (d, J = 8.4 Hz, 2H), 7.42 (t, J = 8.0 Hz, 2H), 7.18 (t, J = 7.2 Hz, 1H), 6.97–7.08 (m, 4H), 3.24 (q, J = 6.6 Hz, 2H), 2.25 (t, J = 7.4 Hz, 2H), 1.72 (tt, J = 7.4, 6.6 Hz, 2H).

4.10. *N*-[4-(Hydroxyamino)-4-oxobutyl]-4-phenoxybenzamide (3)

The title compound was obtained as a white powder in 59% yield from **32** according to the same procedures as described for the preparation of **2**: TLC R_f = 0.25 ($CHCl_3$–MeOH–H_2O–AcOH, 100:10:1:1); MS (MALDI, pos.) m/z 315 $(M+H)^+$; IR (KBr) 1633, 1544, 1489, 1370, 1303, 1258, 1165, 1100, 1028, 972 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ 10.36 (br s, 1H), 8.69 (br s, 1H), 8.41 (t, J = 5.6 Hz, 1H), 7.85 (dt, J = 8.8, 2.8 Hz, 2H), 7.37–7.46 (m, 2H), 7.19 (tt, J = 7.2, 1.2 Hz, 1H), 6.97–7.09 (m, 4H), 3.22 (dt, J = 6.6, 5.6 Hz, 2H), 1.99 (t, J = 7.6 Hz, 2H), 1.71 (tt, J = 7.6, 6.6 Hz, 2H); HRMS (FAB) calcd for $C_{17}H_{19}N_2O_4$: 315.1345. Found: 315.1342.

4.11. Methyl 4-(3-methoxy-1-propynyl)benzoate (34)

The title compound was obtained quantitatively from methyl 4-iodobenzoate (**33**) and 3-methoxy-1-propyne according to the same procedures as described for the preparation of **26**. Purification was performed by silica gel chromatography with EtOAc–*n*-hexane, 1:9, as an eluent: TLC R_f = 0.51 (EtOAc–*n*-hexane, 1:4); ^1H NMR (300 MHz, $CDCl_3$) δ 7.97 (d, J = 8.7 Hz, 2H), 7.49 (d, J = 8.7 Hz, 2H), 4.34 (s, 2H), 3.91 (s, 3H), 3.46 (s, 3H).

4.12. 4-(3-Methoxy-1-propynyl)benzoic acid (35) and ethyl 4-{[4-(3-methoxy-1-propynyl)benzoyl]amino}butanoate (36)

The carboxylic acid **35** was obtained in 79% yield from **34** according to the same procedures as described for the preparation of **27**. The ester **36** was obtained as a white powder in 79% yield from **35** according to the same procedures as described for the preparation of **28**. Purification was performed by silica gel chromatography with $CHCl_3$–EtOAc–AcOH, 90:10:3, as an eluent: MS (MALDI, pos.) m/z 342 $(M+K)^+$, 326 $(M+Na)^+$, 304 $(M+H)^+$; ^1H NMR (200 MHz, $CDCl_3$) δ 7.74 (d, J = 8.6 Hz, 2H), 7.51 (d, J = 8.6 Hz, 2H), 6.65 (m, 1H), 4.34 (s, 2H), 4.13 (q, J = 7.2 Hz, 2H), 3.51 (q, J = 6.8 Hz, 2H), 3.46 (s, 3H), 2.45 (t, J = 6.8 Hz, 2H), 1.97 (quintet, J = 6.8 Hz, 2H), 1.24 (t, J = 7.2 Hz, 3H).

4.13. 4-{[4-(3-Methoxy-1-propynyl)benzoyl]amino}butanoic acid (37)

The title compound was obtained as a white powder in 99% yield from **36** according to the same procedures as described for the preparation of **29**: MS (MALDI, pos.) m/z 314 $(M+K)^+$, 298 $(M+Na)^+$, 276 $(M+H)^+$; ^1H NMR (200 MHz, DMSO-d_6) δ12.03 (br s, 1H), 8.54 (t, J = 5.3 Hz, 1H), 7.85 (d, J = 8.2 Hz, 2H), 7.52 (d, J = 8.2 Hz, 2H), 4.34 (s, 2H), 3.35 (s, 3H), 3.34–3.22 (m, 2H), 2.28 (t, J = 7.0 Hz, 2H), 1.76 (quintet, J = 7.0 Hz, 2H).

4.14. *N*-[4-(Hydroxyamino)-4-oxobutyl]-4-(3-methoxy-1-propynyl)benzamide (4)

The title compound was obtained as a white powder in 71% yield from **37** according to the same procedures as described for the preparation of **2**: TLC R_f = 0.32 ($CHCl_3$–MeOH–AcOH, 18:2:1); MS (MALDI, pos.) m/z 313 $(M+Na)^+$, 291 $(M+H)^+$; IR (KBr) 1633, 1536, 1453, 1365, 1303, 1095 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ 10.39 (s, 1H), 8.57 (t, J = 5.5 Hz, 1H), 7.85 (d, J = 8.6 Hz, 2H), 7.53 (d, J = 8.6 Hz, 2H), 4.35 (s, 2H), 3.35 (s, 3H), 3.25 (dt, J = 7.2, 5.5 Hz, 2H), 2.02 (t, J = 7.2 Hz, 2H), 1.74 (quintet, J = 7.2 Hz, 2H); HRMS (FAB) calcd for $C_{15}H_{19}N_2O_4$: 291.1345. Found: 291.1347.

4.15. (5*S*)-5-(2-Hydroxyethyl)-2,2-dimethyl-1,3-dioxolan-4-one (39a)

To a stirred solution of **38a** (29.4 g, 169 mmol) in THF (300 mL) was added dropwise a solution of borane–tetrahydrofuran complex (203 mL of a 1.0 M solution in THF, 203 mmol) at 0 °C. After being stirred at room temperature for 4 h, the reaction mixture was quenched with saturated NH_4Cl and extracted with EtOAc. The organic layer was washed with brine and dried over Na_2SO_4. Removal of the volatiles in vacuo provided 20.2 g (75%) of **39a** as a yellow oil: TLC $R_f = 0.44$ (EtOAc–*n*-hexane, 1:1); ^1H NMR (200 MHz, $CDCl_3$) δ 4.58 (dd, $J = 6.8$, 5.2 Hz, 1H), 3.95–3.77 (m, 2H), 2.28–1.93 (m, 2H), 1.64 (s, 3H), 1.57 (s, 3H).

4.16. (5*R*)-5-(2-Hydroxyethyl)-2,2-dimethyl-1,3-dioxolan-4-one (39b)

The title compound was obtained as a yellow oil in 89% yield from **38b** according to the same procedures as described for the preparation of **39a**: TLC $R_f = 0.44$ (EtOAc–*n*-hexane, 1:1); ^1H NMR (200 MHz, $CDCl_3$) δ 4.58 (dd, $J = 6.8$, 5.2 Hz, 1H), 3.95–3.77 (m, 2H), 2.28–1.93 (m, 2H), 1.64 (s, 3H), 1.57 (s, 3H).

4.17. 2-[(4*S*)-2,2-Dimethyl-5-oxo-1,3-dioxolan-4-yl]ethyl 4-methylbenzenesulfonate (40a)

To a stirred solution of **39a** (19.8 g, 124 mmol) in pyridine (160 mL) at 0 °C was added *p*-toluenesulfonyl chloride (25.9 g, 136 mmol). After being stirred at room temperature overnight, the reaction mixture was diluted with water and extracted with EtOAc. The organic layer was washed with 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, 1:3, as an eluent. Evaporation of the product-rich fractions provided 24.2 g (61%) of **40a** as a brown oil: TLC $R_f = 0.33$ (EtOAc–*n*-hexane, 1:3); ^1H NMR (200 MHz, $CDCl_3$) δ 7.81 (d, $J = 8.4$ Hz, 2H), 7.36 (d, $J = 8.4$ Hz, 2H), 4.45–4.40 (m, 1H), 4.31–4.10 (m, 2H), 2.46 (s, 3H), 2.35–2.18 (m 1H), 2.10–1.92 (m, 1H), 1.57 (s, 3H), 1.51 (s, 3H).

4.18. 2-[(4*R*)-2,2-Dimethyl-5-oxo-1,3-dioxolan-4-yl]ethyl 4-methylbenzenesulfonate (40b)

The title compound was obtained as a light brown oil in 29% yield from **39b** according to the same procedures as described for the preparation of **40a**: TLC $R_f = 0.33$ (EtOAc–*n*-hexane, 1:3); ^1H NMR (200 MHz, $CDCl_3$) δ 7.81 (d, $J = 8.4$ Hz, 2H), 7.36 (d, $J = 8.4$ Hz, 2H), 4.45–4.40 (m, 1H), 4.31–4.10 (m, 2H), 2.46 (s, 3H), 2.35–2.18 (m 1H), 2.10–1.92 (m, 1H), 1.57 (s, 3H), 1.51 (s, 3H).

4.19. (5*S*)-5-(2-Azidoethyl)-2,2-dimethyl-1,3-dioxolan-4-one (41a)

A solution of **40a** (23.1 g, 73.6 mmol) and sodium azide (5.26 g, 80.9 mmol) in DMF (160 mL) was stirred at 80 °C for 20 min. The reaction mixture was poured into water and extracted with EtOAc. The organic layer was washed with 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, 1:6, as an eluent. Evaporation of the product-rich fractions provided 11.5 g (85%) of **41a** as a yellow oil: TLC $R_f = 0.68$ (EtOAc–*n*-hexane, 1:3); ^1H NMR (200 MHz, $CDCl_3$) δ 4.49 (dd, $J = 7.2$, 4.8 Hz, 1H), 3.54–3.47 (m, 2H), 2.25–1.90 (m, 2H), 1.63 (s, 3H), 1.56 (s, 3H).

4.20. (5*R*)-5-(2-Azidoethyl)-2,2-dimethyl-1,3-dioxolan-4-one (41b)

The title compound was obtained as a yellow oil in 75% yield from **40b** according to the same procedures as described for the preparation of **41a**: TLC $R_f = 0.68$ (EtOAc–*n*-hexane, 1:3); ^1H NMR (200 MHz, $CDCl_3$) δ 4.49 (dd, $J = 7.2$, 4.8 Hz, 1H), 3.54–3.47 (m, 2H), 2.25–1.90 (m, 2H), 1.63 (s, 3H), 1.56 (s, 3H).

4.21. Methyl (2*S*)-4-azido-2-hydroxybutanoate (42a)

A solution of **41a** (1.50 g, 8.10 mmol) and *p*-toluenesulfonic acid monohydrate (69.7 mg, 0.405 mmol) in MeOH (100 mL) was refluxed for 1.5 h. The reaction mixture was evaporated. The residue was diluted with EtOAc and washed with saturated $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4. Removal of the volatiles in vacuo provided 945 mg (74%) of **42a** as a yellow oil: TLC $R_f = 0.50$ (EtOAc–*n*-hexane, 1:3); ^1H NMR (200 MHz, DMSO-d_6) δ 5.60 (d, $J = 6.0$ Hz, 1H), 4.16–4.07 (m, 1H), 3.64 (s, 3H), 3.41 (t, $J = 6.6$ Hz, 2H), 1.98–1.68 (m, 2H).

4.22. Methyl (2*R*)-4-azido-2-hydroxybutanoate (42b)

The title compound was obtained as a yellow oil in 83% yield from **41b** according to the same procedures as described for the preparation of **42a**: TLC $R_f = 0.50$ (EtOAc–*n*-hexane, 1:3); ^1H NMR (200 MHz, DMSO-d_6) δ 5.60 (d, $J = 6.0$ Hz, 1H), 4.16–4.07 (m, 1H), 3.64 (s, 3H), 3.41 (t, $J = 6.6$ Hz, 2H), 1.98–1.68 (m, 2H).

4.23. 4-(1-Benzofuran-2-yl)benzoyl chloride (43)

A suspension of **27** (13.4 g, 56.3 mmol) in $SOCl_2$ (80 mL) was refluxed for 3 h, and the reaction mixture was evaporated. The resulting oily residue was diluted with toluene and the volatiles were removed in vacuo. The resulting solid was washed with Et_2O–*n*–hexane to give 12.7 g (88%) of **43** as a yellow powder: ^1H NMR (200 MHz, $CDCl_3$) δ 8.19 (d, $J = 8.8$ Hz, 2H), 7.98 (d, $J = 8.8$ Hz, 2H), 7.68–7.61 (m, 1H), 7.59–7.53 (m, 1H), 7.42–7.23 (m, 3H).

4.24. Methyl (2*S*)-4-{[4-(1-benzofuran-2-yl)benzoyl]amino}-2-hydroxybutanoate (44a)

Catalytic hydrogenation of **42a** (900 mg, 5.73 mmol) in MeOH–4 N HCl/MeOH (90 mL–2.86 mL) was conducted for 2 h at room temperature in the presence of 10% palladium on carbon (90 mg) at an atmospheric pressure. The catalyst was removed by filtration and

the volatiles were removed in vacuo. The resulting residue was used for the next reaction without further purification. To a stirred solution of the residue in CH_2Cl_2 (20 mL) were added triethylamine (2.4 mL, 17.2 mmol) at 0 °C and then 4-(1-benzofuran-2-yl)benzoyl chloride **(43)** (1.47 g, 5.73 mmol). After being stirred at room temperature for 2 h, the reaction mixture was poured into 1 N HCl and extracted with EtOAc. The organic layer was washed with water, brine and dried over Na_2SO_4. Removal of the volatiles in vacuo provided a solid, which was washed with EtOAc–*n*-hexane to give 1.61 g (80%) of **44a** as a beige powder: TLC $R_f = 0.20$ (EtOAc–*n*-hexane, 2:1); ^{1}H NMR (200 MHz, DMSO-d_6) δ 8.58 (t, $J = 6.0$ Hz, 1H), 8.01 (d, $J = 8.8$ Hz, 2H), 7.96 (d, $J = 8.8$ Hz, 2H), 7.71–7.63 (m, 2H), 7.57 (br s, 1H), 7.39–7.24 (m, 2H), 4.19–4.10 (m, 1H), 3.62 (s, 3H), 3.38 (q, $J = 6.0$ Hz, 2H), 2.06–1.68 (m, 2H).

4.25. Methyl (2*R*)-4-{[4-(1-benzofuran-2-yl)benzoyl]amino}-2-hydroxybutanoate (44b)

The title compound was obtained as a beige powder in 63% yield from **42b** according to the same procedures as described for the preparation of **44a**: TLC $R_f = 0.20$ (EtOAc–*n*-hexane, 2:1); ^{1}H NMR (200 MHz, DMSO-d_6): δ 8.58 (t, $J = 6.0$ Hz, 1H), 8.01 (d, $J = 8.8$ Hz, 2H), 7.96 (d, $J = 8.8$ Hz, 2H), 7.71–7.63 (m, 2H), 7.57 (br s, 1H), 7.39–7.24 (m, 2H), 4.19–4.10 (m, 1H), 3.62 (s, 3H), 3.38 (q, $J = 6.0$ Hz, 2H), 2.06–1.68 (m, 2H).

4.26. (2*S*)-4-{[4-(1-Benzofuran-2-yl)benzoyl]amino}-2-hydroxybutanoic acid (45a)

The title compound was obtained as an off-white powder in 77% yield from **44a** according to the same procedures as described for the preparation of **29**: TLC $R_f = 0.10$ ($CHCl_3$–MeOH–AcOH, 100:10:1); ^{1}H NMR (200 MHz, DMSO-d_6) δ 8.49 (t, $J = 5.6$ Hz, 1H), 7.92 (d, $J = 8.8$ Hz, 2H), 7.87 (d, $J = 8.4$ Hz, 2H), 7.62–7.53 (m, 2H), 7.48 (d, $J = 0.6$ Hz, 1H), 7.30–7.14 (m, 2H), 3.95 (dd, $J = 8.4$, 4.4 Hz, 1H), 3.29 (q, $J = 6.6$ Hz, 2H), 1.98–1.58 (m, 2H).

4.27. (2*R*)-4-{[4-(1-Benzofuran-2-yl)benzoyl]amino}-2-hydroxybutanoic acid (45b)

The title compound was obtained as a white powder in 93% from **44b** yield according to the same procedures as described for the preparation of **29**: TLC $R_f = 0.10$ ($CHCl_3$–MeOH–AcOH, 100:10:1); ^{1}H NMR (200 MHz, DMSO-d_6) δ 8.49 (t, $J = 5.6$ Hz, 1H), 7.92 (d, $J = 8.8$ Hz, 2H), 7.87 (d, $J = 8.4$ Hz, 2H), 7.62–7.53 (m, 2H), 7.48 (d, $J = 0.6$ Hz, 1H), 7.30–7.14 (m, 2H), 3.95 (dd, $J = 8.4$, 4.4 Hz, 1H), 3.29 (q, $J = 6.6$ Hz, 2H), 1.98–1.58 (m, 2H).

4.28. 4-(1-Benzofuran-2-yl)-*N*-[(3*S*)-3-hydroxy-4-(hydroxyamino)-4-oxobutyl]benzamide (5)

The title compound was obtained as a white powder in 86% yield from **45a** according to the same procedures as described for the preparation of **2**: TLC $R_f = 0.42$ ($CHCl_3$–MeOH–H_2O, 100:20:1); MS (MALDI, pos.) *m*/*z* 377 $(M+Na)^+$, 355 $(M+H)^+$; IR (KBr) 1635, 1539, 1497, 1450, 1306, 1107, 1062 cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ 10.49 (br s, 1H), 8.55 (t, $J = 5.4$ Hz, 1H), 8.01 (d, $J = 9.0$ Hz, 2H), 7.96 (d, $J = 9.0$ Hz, 2H), 7.71–7.62 (m, 2H), 7.57 (br s, 1H), 7.39–7.23 (m, 2H), 5.00–3.60 (br, 1H), 3.94 (dd, $J = 8.2$, 4.2 Hz, 1H), 3.41–3.31 (m, 2H), 2.02–1.64 (m, 2H); optical rotation $[\alpha]_D^{30}$ −11.5 (*c* 0.38, DMF); HRMS (FAB) calcd for $C_{19}H_{19}N_2O_5$: 355.1294. Found: 355.1294.

4.29. 4-(1-Benzofuran-2-yl)-*N*-[(3*R*)-3-hydroxy-4-(hydroxyamino)-4-oxobutyl]benzamide (6)

The title compound was obtained as a white powder in 84% yield from **45b** according to the same procedures as described for the preparation of **2**: TLC $R_f = 0.42$ ($CHCl_3$–MeOH–H_2O, 100:20:1); MS (MALDI, pos.) *m*/*z* 355 $(M+H)^+$; IR (KBr) 1634, 1539, 1497, 1450, 1306, 1108, 1062 cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ 10.49 (br s, 1H), 8.55 (t, $J = 5.6$ Hz, 1H), 8.01 (d, $J = 9.0$ Hz, 2H), 7.96 (d, $J = 9.0$ Hz, 2H), 7.71–7.63 (m, 2H), 7.57 (br s, 1H), 7.39–7.23 (m, 2H), 6.00–4.20 (br, 1H), 3.94 (dd, $J = 8.2$, 4.2 Hz, 1H), 3.41–3.31 (m, 2H), 2.02–1.62 (m, 2H); optical rotation $[\alpha]_D^{30}$ +10.4 (*c* 0.44, DMF); HRMS (FAB) calcd for $C_{19}H_{19}N_2O_5$: 355.1294. Found: 355.1295.

4.30. Methyl (2*S*)-4-{[4-(1-benzofuran-2-yl)benzoyl]amino}-2-[(benzyloxy)methoxy]butanoate (46a)

To a stirred solution of **44a** (200 mg, 0.566 mmol) in CH_2Cl_2 (1 mL) were added *N*,*N*-diisopropylethylamine (2 mL) at 0 °C and then benzyloxymethylchloride (784 μL, 5.66 mmol). After being stirred at 50 °C for 30 min, the reaction mixture was poured into 1 N HCl and extracted with EtOAc. The organic layer was washed with water, 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, 2:3, as an eluent. Evaporation of the product-rich fractions provided 176 mg (66%) of **46a** as a white powder: TLC $R_f = 0.47$ (EtOAc–*n*-hexane, 1:1); ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.89 (d, $J = 8.8$ Hz, 2H), 7.79 (d, $J = 8.8$ Hz, 2H), 7.64–7.52 (m, 2H), 7.37–7.21 (m, 9H), 7.12 (br s, 1H), 4.92 (d, $J = 7.0$ Hz, 1H), 4.87 (d, $J = 7.0$ Hz, 1H), 4.68 (s, 2H), 4.38 (dd, $J = 6.0$, 5.0 Hz, 1H), 2.20–2.10 (m, 2H); optical rotation $[\alpha]_D^{30}$ −42.4 (*c* 0.40, $CHCl_3$).

4.31. Methyl (2*R*)-4-{[4-(1-benzofuran-2-yl)benzoyl]amino}-2-[(benzyloxy)methoxy]butanoate (46b)

The title compound was obtained as a white powder in 82% yield from **44b** according to the same procedures as described for the preparation of **46a**: TLC $R_f = 0.47$ (EtOAc–*n*-hexane, 1:1); ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.89 (d, $J = 8.8$ Hz, 2H), 7.79 (d, $J = 8.8$ Hz, 2H), 7.64–7.52 (m, 2H), 7.37–7.21 (m, 9H), 7.12 (br s, 1H), 4.92 (d, $J = 7.0$ Hz, 1H), 4.87 (d, $J = 7.0$ Hz, 1H), 4.68 (s, 2H), 4.38 (dd, $J = 6.0$, 5.0 Hz, 1H), 2.20–2.10 (m, 2H).

4.32. (2*S*)-4-{[4-(1-Benzofuran-2-yl)benzoyl]amino}-2-[(benzyloxy)methoxy]butanoic acid (47a)

The title compound was obtained as a white powder in 96% yield from **46a** according to the same procedures as described for the preparation of **29**: TLC $R_f = 0.45$ ($CHCl_3$–MeOH–H_2O, 100:20:1); ^1H NMR (200 MHz, DMSO-d_6) δ 8.61 (t, $J = 5.4$ Hz, 1H), 8.01 (d, $J = 8.8$ Hz, 2H), 7.95 (d, $J = 8.8$ Hz, 2H), 7.70–7.63 (m, 2H), 7.57 (br s, 1H), 4.82 (d, $J = 7.0$ Hz, 1H), 4.77 (d, $J = 7.0$ Hz, 1H), 4.64 (d, $J = 11.8$ Hz, 1H), 4.54 (d, $J = 11.8$ Hz, 1H), 4.13 (dd, $J = 8.2$, 4.2 Hz, 1H), 3.52–3.28 (m, 2H), 2.12–1.82 (m, 2H).

4.33. (2*R*)-4-{[4-(1-Benzofuran-2-yl)benzoyl]amino}-2-[(benzyloxy)methoxy]butanoic acid (47b)

The title compound was obtained as a white powder in 100% yield from **46b** according to the same procedures as described for the preparation of **29**: TLC $R_f = 0.45$ ($CHCl_3$–MeOH–H_2O, 100:20:1); ^1H NMR (200 MHz, DMSO-d_6) δ 8.61 (t, $J = 5.4$ Hz, 1H), 8.01 (d, $J = 8.8$ Hz, 2H), 7.95 (d, $J = 8.8$ Hz, 2H), 7.70–7.63 (m, 2H), 7.57 (br s, 1H), 4.82 (d, $J = 7.0$ Hz, 1H), 4.77 (d, $J = 7.0$ Hz, 1H), 4.64 (d, $J = 11.8$ Hz, 1H), 4.54 (d, $J = 11.8$ Hz, 1H), 4.13 (dd, $J = 8.2$, 4.2 Hz, 1H), 3.52–3.28 (m, 2H), 2.12–1.82 (m, 2H).

4.34. 4-(1-Benzofuran-2-yl)-*N*-[(3*S*)-3-[(benzyloxy)methoxy]-4-(hydroxyamino)-4-oxobutyl]benzamide (7)

The title compound was obtained as a white powder in 62% yield from **47a** according to the same procedures as described for the preparation of **2**: TLC $R_f = 0.24$ ($CHCl_3$–MeOH, 10:1); MS (APCI, neg. 40 V) *m/z* 473 $(M-H)^-$; IR (KBr) 1627, 1539, 1497, 1450, 1310, 1108, 1065, 1032 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ 10.75 (br s, 1H), 8.90 (br s, 1H), 8.55 (t, $J = 5.6$ Hz, 1H), 8.01 (d, $J = 8.8$ Hz, 2H), 7.96 (d, $J = 8.8$ Hz, 2H), 7.71–7.63 (m, 2H), 7.58 (br s, 1H), 7.39–7.24 (m, 7H), 4.77 (d, $J = 7.0$ Hz, 1H), 4.68 (d, $J = 7.0$ Hz, 1H), 4.58 (s, 2H), 4.04 (t, $J = 5.8$ Hz, 1H), 3.49–3.25 (m, 2H), 1.93 (q, $J = 6.2$ Hz, 2H); optical rotation $[\alpha]_D^{30}$ −27.0 (*c* 0.23, DMF); HRMS (FAB) calcd for $C_{27}H_{27}N_2O_6$: 475.1869. Found: 475.1867.

4.35. 4-(1-Benzofuran-2-yl)-*N*-[(3*R*)-3-[(benzyloxy)methoxy]-4-(hydroxyamino)-4-oxobutyl]benzamide (8)

The title compound was obtained as a white powder in 61% yield from **47b** according to the same procedures as described for the preparation of **2**: TLC $R_f = 0.24$ ($CHCl_3$–MeOH, 10:1); MS (APCI, neg. 40 V) *m/z* 473 $(M-H)^-$; IR (KBr) 1627, 1539, 1497, 1450, 1311, 1108, 1065, 1031 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ 10.75 (br s, 1H), 8.90 (br s, 1H), 8.55 (t, $J = 5.6$ Hz, 1H), 8.01 (d, $J = 8.8$ Hz, 2H), 7.96 (d, $J = 8.8$ Hz, 2H), 7.71–7.63 (m, 2H), 7.58 (br s, 1H), 7.39–7.24 (m, 7H), 4.77 (d, $J = 7.0$ Hz, 1H), 4.68 (d, $J = 7.0$ Hz, 1H), 4.58 (s, 2H), 4.04 (t, $J = 5.8$ Hz, 1H), 3.49–3.25 (m, 2H), 1.93 (q, $J = 6.2$ Hz, 2H); optical rotation $[\alpha]_D^{30}$ +25.2 (*c* 0.23, DMF); HRMS (FAB) calcd for $C_{27}H_{27}N_2O_6$: 475.1869. Found: 475.1872.

4.36. Ethyl 4-[(4-iodobenzoyl)amino]butanoate (49) and ethyl 2-benzyl-4-[(4-iodobenzoyl)amino]butanoate (50)

The ester **49** was obtained as a white powder in 75% yield from **48** according to the same procedures as described for the preparation of **28**. To a stirred solution of **49** (410 mg, 1.14 mmol) in THF (10 mL) was added dropwise LiHMDS (2.51 mL of a 1.0 M solution in THF) at −78 °C. The reaction mixture was stirred for 1 h. After dropwise addition of benzyl bromide (407 μL, 3.42 mmol), the reaction mixture was warmed up to −30 °C, stirred for 3 h, quenched with saturated NH_4Cl, and extracted with EtOAc. The organic layer was washed with brine and dried over Na_2SO_4. Removal of the volatiles in vacuo provided a solid, which was washed with Et_2O–*n*-hexane to give 424 mg (82%) of **50** as a white powder: TLC $R_f = 0.69$ (EtOAc–*n*-hexane, 1:1); ^1H NMR (200 MHz, DMSO-d_6) δ 8.53 (t, $J = 5.6$ Hz, 1H), 7.84 (d, $J = 8.4$ Hz, 2H), 7.59 (d, $J = 8.4$ Hz, 2H), 7.30–7.12 (m, 5H), 3.94 (q, $J = 7.0$ Hz, 2H), 3.33–3.20 (m, 2H), 2.86–2.62 (m, 3H), 1.90–1.60 (m, 2H), 1.02 (t, $J = 7.0$ Hz, 3H).

4.37. Ethyl 4-{[4-(1-benzofuran-2-yl)benzoyl]amino}-2-benzylbutanoate (51)

The title compound was obtained as an off-white powder in 81% yield from **50** according to the same procedures as described for the preparation of **26**: TLC $R_f = 0.47$ (EtOAc–*n*-hexane, 1:2); ^1H NMR (200 MHz, $CDCl_3$): δ 7.92 (d, $J = 8.8$ Hz, 2H), 7.82 (d, $J = 8.8$ Hz, 2H), 7.63–7.52 (m, 2H), 7.36–7.16 (m, 7H), 7.12 (br s, 1H), 6.41 (m, 1H), 4.05 (br q, $J = 7.0$ Hz, 2H), 3.62–3.41 (m, 2H), 3.09–2.96 (m, 1H), 2.87–2.72 (m, 2H), 1.13 (t, $J = 7.0$ Hz, 3H).

4.38. 4-{[4-(1-Benzofuran-2-yl)benzoyl]amino}-2-benzylbutanoic acid (52)

The title compound was obtained as a white powder in 86% yield from **51** according to the same procedures as described for the preparation of **29**: TLC $R_f = 0.37$ ($CHCl_3$–MeOH, 10:1); ^1H NMR (200 MHz, DMSO-d_6) δ 12.22 (br s, 1H), 8.58 (t, $J = 5.6$ Hz, 1H), 7.70–7.63 (m, 2H), 7.57 (br s, 1H), 7.39–7.14 (m, 7H), 3.39–3.25 (m, 2H), 2.92–2.62 (m, 3H), 1.90–1.60 (m, 2H).

4.39. 4-(1-Benzofuran-2-yl)-*N*-[3-benzyl-4-(hydroxyamino)-4-oxobutyl]benzamide (9)

The title compound was obtained as a white powder in 65% yield from **52** according to the same procedures as described for the preparation of **2**: TLC $R_f = 0.39$ ($CHCl_3$–MeOH, 10:1); MS (MALDI, pos.) *m/z* 467 $(M+K)^+$, 451 $(M+Na)^+$, 429 $(M+H)^+$; IR (KBr) 1655, 1636, 1607, 1560, 1497, 1451, 1308, 1257, 1200, 1171, 1029 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ 10.43 (s, 1H), 8.52 (t, $J = 5.6$ Hz, 1H), 8.00 (d, $J = 8.8$ Hz, 2H), 7.94 (d, $J = 8.8$ Hz, 2H), 7.71–7.63 (m, 2H), 7.57 (s, 1H), 7.39–7.16 (m, 7H), 3.30–3.15 (m, 2H), 2.83 (dd, $J = 13.6$, 8.6 Hz, 1H), 2.66 (dd, $J = 13.6$, 6.2 Hz, 1H), 2.44–2.31 (m, 1H), 1.88–1.53

(m, 2H); HRMS (FAB) calcd for $C_{26}H_{25}N_2O_4$: 429.1814. Found: 429.1811.

4.40. Ethyl 2-(cyanomethyl)-4-phenylbutanoate (54)

To a stirred solution of ethyl 4-phenylbutanoate **(53)** (500 mg, 2.60 mmol) in THF (10 mL) was added dropwise LDA (1.30 mL of a 2.0 M solution in THF, 2.60 mmol) at −78 °C. The reaction mixture was stirred for 30 min, and bromoacetonitrile (362 μL, 5.20 mmol) was added dropwise. After being stirred at −78 °C for 1 h, the reaction mixture was quenched with saturated NH_4Cl and extracted with EtOAc. The organic layer was washed with water, 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, 1:8, as an eluent. Evaporation of the product-rich fractions provided 270 mg (45%) of **54**: TLC $R_f = 0.25$ (EtOAc–*n*-hexane, 1:6); ^1H NMR (200 MHz, $CDCl_3$) δ 7.37–7.18 (m, 5H), 4.21 (q, $J = 7.0$ Hz, 2H), 2.82–2.50 (m, 5H), 2.23–1.89 (m, 2H), 1.31 (t, $J = 7.0$ Hz, 3H).

4.41. Ethyl 4-amino-2-(2-phenylethyl)butanoate hydrochloride (55) and ethyl 4-{[4-(1-benzofuran-2-yl)benzoyl]amino}-2-(2-phenylethyl)butanoate (56)

Catalytic hydrogenation of **54** (218 mg, 0.943 mmol) in MeOH-4 N HCl/EtOAc (15 mL-0.71 mL) was conducted for 1.5 h at room temperature in the presence of 10% PtO_2 (10 mg) at an atmospheric pressure. Removal of the catalyst by filtration, followed by evaporation of the solvent, provided **55** as an oily residue. To a stirred solution of **55** (0.943 mmol) in CH_2Cl_2 (10 mL) were added triethylamine (525 μL, 3.77 mmol) at 0 °C and then 4-(1-benzofuran-2-yl)benzoyl chloride (**43**) (242 mg, 0.943 mmol). After being stirred at room temperature for 30 min, the reaction mixture was poured into 1 N HCl and extracted with EtOAc. The organic layer was washed with water, brine and dried over Na_2SO_4. Removal of the volatiles in vacuo provided an oily residue, which was purified by silica gel chromatography using EtOAc–*n*-hexane, 1:3, as an eluent. Evaporation of the product-rich fractions provided 212 mg (49% in two steps) of **56** as a white powder: TLC $R_f = 0.71$ (EtOAc–*n*-hexane, 1:1); ^1H NMR (200 MHz, $CDCl_3$) δ 7.92 (d, $J = 8.8$ Hz, 2H), 7.82 (d, $J = 8.8$ Hz, 2H), 7.63–7.52 (m, 2H), 7.36–7.15 (m, 7H), 7.12 (br s, 1H), 4.15 (q, $J = 7.0$ Hz, 2H), 3.65–3.38 (m, 2H), 2.68–2.45 (m, 3H), 2.15–1.74 (m, 4H), 1.26 (t, $J = 7.0$ Hz, 3H).

4.42. 4-{[4-(1-Benzofuran-2-yl)benzoyl]amino}-2-(2-phenylethyl)butanoic acid (57)

The title compound was obtained as a white powder in 88% yield from **56** according to the same procedures as described for the preparation of **29**: TLC $R_f = 0.42$ ($CHCl_3$–MeOH, 10:1); (200 MHz, DMSO-d_6) δ 8.57 (br t, $J = 5.6$ Hz, 1H), 8.01 (d, $J = 8.8$ Hz, 2H), 7.95 (d, $J = 8.8$ Hz, 2H), 7.71–7.63 (m, 2H), 7.58 (br s, 1H), 7.40–7.12 (m, 7H), 3.35–3.25 (m, 2H), 2.57 (t, $J = 7.8$ Hz, 2H), 2.42–2.28 (m, 1H), 1.94–1.65 (m, 4H).

4.43. 4-(1-Benzofuran-2-yl)-*N*-{3-[(hydroxyamino)carbonyl]-5-phenylpentyl}benzamide (10)

The title compound was obtained as a white powder in 59% yield from **57** according to the same procedures as described for the preparation of **2**: TLC $R_f = 0.47$ ($CHCl_3$–MeOH, 10:1); MS (MALDI, pos.) *m*/*z* 481 $(M+K)^+$, 465 $(M+Na)^+$, 443 $(M+H)^+$; IR (KBr) 1632, 1540, 1496, 1451, 1307, 1258, 1170, 1030 cm^{-1}; ^1H NMR (200 MHz, CD_3OD) δ 7.98 (d, $J = 8.4$ Hz, 2H), 7.89 (d, $J = 8.4$ Hz, 2H), 7.64–7.60 (m, 1H), 7.56–7.52 (m, 1H), 7.36–7.09 (m, 7H), 7.32 (br s, 1H), 3.51–3.24 (m, 2H), 2.68–2.50 (m, 2H), 2.28–2.14 (m, 1H), 2.04–1.69 (m, 4H); HRMS (FAB) calcd for $C_{27}H_{27}N_2O_4$: 443.1971. Found: 443.1973.

4.44. Ethyl (4*E*)-2-{2-[(4-iodobenzoyl)amino]ethyl}-5-phenyl-4-pentenoate (58)

To a stirred solution of ethyl 4-[(4-iodobenzoyl)amino]butanoate (**49**) (800 mg, 2.21 mmol) in THF (20 mL) was added dropwise LiHMDS (4.86 mL of a 1.0 M solution in THF, 4.86 mmol) at −78 °C. The reaction mixture was stirred for 1 h and 1-bromo-3-phenyl-2-propene (1.31 g, 6.63 mmol) was added dropwise. The reaction mixture was warmed up to −40 °C, stirred for 2 h, quenched with saturated NH_4Cl, and extracted with EtOAc. The organic layer was washed with water, brine and dried over Na_2SO_4. Removal of the volatiles in vacuo provided a solid, which was washed with Et_2O–*n*-hexane to give 700 mg (67%) of **58** as a white powder: TLC $R_f = 0.44$ (EtOAc–*n*-hexane, 1:2); ^1H NMR (200 MHz, $CDCl_3$) δ 7.78 (d, $J = 8.7$ Hz, 2H), 7.49 (d, $J = 8.7$ Hz, 2H), 7.38–7.15 (m, 5H), 6.43 (d, $J = 15.6$ Hz, 1H), 6.39 (br s, 1H), 6.12 (dt, $J = 15.6$, 6.9 Hz, 1H), 4.21–4.02 (m, 2H), 3.62–3.40 (m, 2H), 2.70–2.38 (m, 3H), 2.05–1.82 (m, 2H), 1.21 (t, $J = 7.2$ Hz, 3H).

4.45. Ethyl (4*E*)-2-(2-{[4-(1-benzofuran-2-yl)benzoyl]amino}ethyl)-5-phenyl-4-pentenoate (59)

The title compound was obtained as a gray powder in 95% yield from **58** according to the same procedures as described for the preparation of **26**: TLC $R_f = 0.38$ (EtOAc–*n*-hexane, 1:2); ^1H NMR (200 MHz, $CDCl_3$) δ 7.92 (d, $J = 8.8$ Hz, 2H), 7.85 (d, $J = 8.8$ Hz, 2H), 7.63–7.52 (m, 2H), 7.36–7.21 (m, 7H), 7.12 (d, $J = 0.8$ Hz, 1H), 6.49 (t, $J = 5.2$ Hz, 1H), 6.45 (d, $J = 15.8$ Hz, 1H), 6.13 (dt, $J = 15.8$, 7.2 Hz, 1H), 4.21–4.05 (m, 2H), 3.66–3.45 (m, 2H), 2.70–2.43 (m, 3H), 2.09–1.85 (m, 2H), 1.22 (t, $J = 7.4$ Hz, 3H).

4.46. Ethyl 2-(2-{[4-(1-benzofuran-2-yl)benzoyl]amino}ethyl)-5-phenylpentanoate (60)

Catalytic hydrogenation of **59** (300 mg, 0.642 mmol) in MeOH (10 mL) and DMF (0.5 mL) was conducted at room temperature for 30 min in the presence of 10% palladium on carbon (30 mg) at an atmospheric pressure. The catalyst was removed by filtration and the volatiles were removed in vacuo. The resulting residue was diluted with water and extracted with EtOAc. The organic

layer was washed with brine and dried over Na_2SO_4. Removal of the volatiles in vacuo provided 300 mg (99%) of **60** as a white powder: TLC $R_f = 0.33$ (EtOAc–*n*-hexane, 1:2); ^1H NMR (200 MHz, $CDCl_3$) δ 7.93 (d, $J = 8.8$ Hz, 2H), 7.84 (d, $J = 8.8$ Hz, 2H), 7.63–7.52 (m, 2H), 7.36–7.13 (m, 7H), 7.12 (br s, 1H), 4.12 (br q, $J = 6.8$ Hz, 2H), 3.63–3.36 (m, 2H), 2.62 (t, $J = 6.8$ Hz, 2H), 2.56–2.44 (m, 1H), 2.05–1.50 (m, 6H), 1.23 (t, $J = 6.8$ Hz, 3H).

4.47. 2-(2-{[4-(1-Benzofuran-2-yl)benzoyl]amino}ethyl)-5-phenylpentanoic acid (61)

The title compound was obtained as a white powder in 96% yield from **60** according to the same procedures as described for the preparation of **29**: TLC $R_f = 0.45$ ($CHCl_3$–MeOH, 10:1); ^1H NMR (200 MHz, DMSO-d_6) δ 12.18 (br s, 1H), 8.55 (t, $J = 5.4$ Hz, 1H), 7.71–7.62 (m, 2H), 7.57 (br s, 1H), 7.39–7.11 (m, 7H), 3.31–3.22 (m, 2H), 2.61–2.53 (m, 2H), 2.46–2.28 (m, 1H), 1.89–1.46 (m, 8H).

4.48. 4-(1-Benzofuran-2-yl)-*N*-{3-[(hydroxyamino)carbonyl]-6-phenylhexyl}benzamide (11)

The title compound was obtained as a white powder in 80% yield from **61** according to the same procedures as described for the preparation of **2**: TLC $R_f = 0.41$ ($CHCl_3$–MeOH, 10:1); MS (MALDI, pos.) *m*/*z* 495 $(M+K)^+$, 479 $(M+Na)^+$, 457 $(M+H)^+$; IR (KBr) 1634, 1542, 1497, 1451, 1307, 1258, 1170, 1031 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ 10.48 (s, 1H), 8.50 (t, $J = 5.6$ Hz, 1H), 8.01 (d, $J = 8.8$ Hz, 2H), 7.95 (d, $J = 8.8$ Hz, 2H), 7.71–7.63 (m, 2H), 7.57 (s, 1H), 7.39–7.11 (m, 7H), 3.25–3.15 (m, 2H), 2.59–2.47 (m, 2H), 2.18–2.02 (m, 1H), 1.82–1.36 (m, 6H); HRMS (FAB) calcd for $C_{28}H_{29}N_2O_4$: 457.2127. Found: 457.2122.

4.49. Methyl (3*S*)-3,4-dihydroxybutanoate (63a) and methyl (3*S*)-3-hydroxy-4-{[(4-methylphenyl)sulfonyl]oxy}butanoate (64a)

To a stirred solution of dimethyl (2*S*)-2-hydroxysuccinate (**62a**) (15.0 g, 92.5 mmol) in THF (170 mL) were added dropwise borane–methyl sulfide complex (7.24 g, 95.3 mmol) at room temperature and then sodium borohydride (175 mg, 4.63 mmol) in one portion at 0 °C. After being stirred at 0 °C for 10 min, the reaction mixture was warmed up to room temperature and stirred for 30 min. To the stirred reaction mixture were added MeOH (40 mL) and *p*-toluenesulfonic acid monohydrate (797 mg, 4.63 mmol) at room temperature. After being stirred for 30 min, the reaction mixture was evaporated. The resulting residue was diluted with MeOH–benzene and the resulting solution was evaporated again. This operation was repeated several times. Dilution of the resulting residue with benzene followed by removal of the volatiles in vacuo provided 13.0 g (100%) of **63a** as a yellow oil: TLC $R_f = 0.25$ (EtOAc). The tosylate **64a** was obtained as a brown solid in 48% yield from **63a** according to the same procedures as described for the preparation of **40a**: TLC $R_f = 0.17$ (EtOAc–*n*-hexane, 1:2); ^1H NMR (200 MHz, $CDCl_3$) δ 7.80 (d, $J = 8.4$ Hz, 2H), 7.36 (d, $J = 8.4$ Hz, 2H), 4.32–4.18 (m, 1H), 4.05 (d, $J = 5.2$ Hz, 2H), 3.70 (s, 3H), 3.03 (br s, 1H), 2.57–2.53 (m, 2H), 2.46 (s, 3H); optical rotation $[\alpha]_D^{30}$ −7.23 (*c* 0.59, $CHCl_3$).

4.50. Methyl (3*R*)-3,4-dihydroxybutanoate (63b) and methyl (3*R*)-3-hydroxy-4-{[(4-methylphenyl)sulfonyl]oxy}butanoate (64b)

The diol **63b** was obtained quantitatively from **62b** as a yellow oil according to the same procedures as described for the preparation of **63a**: TLC $R_f = 0.25$ (EtOAc). The tosylate **64b** was obtained as a brown solid in 50% yield from **63b** according to the same procedures as described for the preparation of **40a**: TLC $R_f = 0.17$ (EtOAc–*n*-hexane, 1:2); ^1H NMR (200 MHz, $CDCl_3$) δ 7.80 (d, $J = 8.4$ Hz, 2H), 7.36 (d, $J = 8.4$ Hz, 2H), 4.32–4.18 (m, 1H), 4.05 (d, $J = 5.2$ Hz, 2H), 3.70 (s, 3H), 3.03 (br s, 1H), 2.57–2.53 (m, 2H), 2.46 (s, 3H); optical rotation $[\alpha]_D^{30}$ +5.46 (*c* 0.505, $CHCl_3$).

4.51. Methyl (3*S*)-4-azido-3-hydroxybutanoate (65a)

The title compound was obtained as a yellow oil in 77% yield from **64a** according to the same procedures as described for the preparation of **41a**: TLC $R_f = 0.68$ (EtOAc–*n*-hexane, 1:1); ^1H NMR (200 MHz, $CDCl_3$) δ 4.28–4.14 (m, 1H), 3.74 (s, 3H), 3.40 (dd, $J = 9.6$, 3.1 Hz, 1H), 3.33 (dd, $J = 9.6$, 4.1 Hz, 1H), 3.14 (d, $J = 4.0$ Hz, 1H), 2.58–2.55 (m, 2H).

4.52. Methyl (3*R*)-4-azido-3-hydroxybutanoate (65b)

The title compound was obtained as a yellow oil in 84% yield from **64b** according to the same procedures as described for the preparation of **41a**: TLC $R_f = 0.68$ (EtOAc–*n*-hexane, 1:1); ^1H NMR (200 MHz, $CDCl_3$) δ 4.28–4.14 (m, 1H), 3.74 (s, 3H), 3.40 (dd, $J = 9.6$, 3.1 Hz, 1H), 3.33 (dd, $J = 9.6$, 4.1 Hz, 1H), 3.14 (d, $J = 4.0$ Hz, 1H), 2.58–2.55 (m, 2H).

4.53. Methyl (3*S*)-4-{[4-(1-benzofuran-2-yl)benzoyl]amino}-3-hydroxybutanoate (66a)

The title compound was obtained as a beige powder in 84% yield from **65a** according to the same procedures as described for the preparation of **44a**: TLC $R_f = 0.31$ (EtOAc–*n*-hexane, 1:1); ^1H NMR (200 MHz, DMSO-d_6) δ 8.57 (t, $J = 6.0$ Hz, 1H), 8.00 (m, 4H), 7.71–7.63 (m, 2H), 7.57 (br s, 1H), 7.40–7.24 (m, 2H), 5.13 (d, $J = 5.6$ Hz, 1H), 4.18–4.00 (m, 1H), 3.57 (s, 3H), 3.30 (t, $J = 6.0$ Hz, 2H), 2.55 (dd, $J = 15.0$, 4.0 Hz, 1H), 2.31 (dd, $J = 15.0$, 4.0 Hz, 1H).

4.54. Methyl (3*R*)-4-{[4-(1-benzofuran-2-yl)benzoyl]amino}-3-hydroxybutanoate (66b)

The title compound was obtained as a beige powder in 74% yield from **65b** according to the same procedures as described for the preparation of **44a**: TLC $R_f = 0.31$ (EtOAc–*n*-hexane, 1:1); ^1H NMR (200 MHz, DMSO-d_6) δ 8.57 (t, $J = 6.0$ Hz, 1H), 8.00 (m, 4H), 7.71–7.63 (m, 2H), 7.57 (br s, 1H), 7.40–7.24 (m, 2H), 5.13 (d,

J = 5.6 Hz, 1H), 4.18–4.00 (m, 1H), 3.57 (s, 3H), 3.30 (t, J = 6.0 Hz, 2H), 2.55 (dd, J = 15.0, 4.0 Hz, 1H), 2.31 (dd, J = 15.0, 4.0 Hz, 1H).

4.55. (3*S*)-4-{[4-(1-Benzofuran-2-yl)benzoyl]amino}-3-hydroxybutanoic acid (67a)

The title compound was obtained as a beige powder in 84% yield from **66a** according to the same procedures as described for the preparation of **29**: TLC R_f = 0.24 ($CHCl_3$–MeOH–AcOH, 100:10:1); ^{1}H NMR (200 MHz, DMSO-d_6) δ 8.55 (t, J = 5.8 Hz, 1H), 7.71–7.63 (m, 2H), 7.58 (br s, 1H), 7.39–7.23 (m, 2H), 5.16–4.92 (br s, 1H), 4.14–4.00 (m, 1H), 3.30 (t, J = 5.8 Hz, 2H), 2.46 (dd, J = 15.0, 4.2 Hz, 1H), 2.23 (dd, J = 15.0, 8.4 Hz, 1H).

4.56. (3*R*)-4-{[4-(1-Benzofuran-2-yl)benzoyl]amino}-3-hydroxybutanoic acid (67b)

The title compound was obtained as a beige powder in 88% yield from **66b** according to the same procedures as described for the preparation of **29**: TLC R_f = 0.24 ($CHCl_3$–MeOH–AcOH, 100:10:1); ^{1}H NMR (200 MHz, DMSO-d_6) δ 8.55 (t, J = 5.8 Hz, 1H), 7.71–7.63 (m, 2H), 7.58 (br s, 1H), 7.39–7.23 (m, 2H), 5.16–4.92 (br s, 1H), 4.14–4.00 (m, 1H), 3.30 (t, J = 5.8 Hz, 2H), 2.46 (dd, J = 15.0, 4.2 Hz, 1H), 2.23 (dd, J = 15.0, 8.4 Hz, 1H).

4.57. 4-(1-Benzofuran-2-yl)-*N*-[(2*S*)-2-hydroxy-4-(hydroxyamino)-4-oxobutyl]benzamide (12)

The title compound was obtained as a white powder in 70% yield from **67a** according to the same procedures as described for the preparation of **2**: TLC R_f = 0.41 ($CHCl_3$–MeOH–H_2O, 100:20:1); MS (MALDI, pos.) m/z 377 $(M+Na)^+$, 355 $(M+H)^+$; IR (KBr) 1636, 1541, 1497, 1450, 1308, 1082 cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ 10.39 (br s, 1H), 8.55 (m, 1H), 8.00 (s, 4H), 7.71–7.63 (m, 2H), 7.57 (br s, 1H), 7.39–7.23 (m, 2H), 5.50–4.30 (br, 1H), 4.12–3.98 (m, 1H), 3.40–3.18 (m, 2H), 2.16 (dd, J = 14.0, 4.8 Hz, 1H), 2.03 (dd, J = 14.0, 8.2 Hz, 1H); optical rotation $[\alpha]_D^{30}$ −4.95 (c 0.42, DMF); HRMS (FAB) calcd for $C_{19}H_{19}N_2O_5$: 355.1294. Found: 355.1290.

4.58. 4-(1-Benzofuran-2-yl)-*N*-[(2*R*)-2-hydroxy-4-(hydroxyamino)-4-oxobutyl]benzamide (13)

The title compound was obtained as a white powder in 53% yield from **67b** according to the same procedures as described for the preparation of **2**: TLC R_f = 0.41 ($CHCl_3$–MeOH–H_2O, 100:20:1); MS (MALDI, pos.) m/z 355 $(M+H)^+$; IR (KBr) 1635, 1541, 1497, 1450, 1307, 1172, 1083, 1034 cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ 10.39 (br s, 1H), 8.55 (t, J = 5.4 Hz, 1H), 8.00 (s, 4H), 7.71–7.63 (m, 2H), 7.58 (br s, 1H), 7.39–7.24 (m, 2H), 5.20–3.80 (br, 1H), 4.12–3.98 (m, 1H), 3.40–3.18 (m, 2H), 2.16 (dd, J = 14.0, 4.8 Hz, 1H), 2.03 (dd, J = 14.0, 8.2 Hz, 1H); optical rotation $[\alpha]_D^{30}$ +3.76 (c 0.41, DMF); HRMS (FAB) calcd for $C_{19}H_{19}N_2O_5$: 355.1294. Found: 355.1290.

4.59. Methyl (3*S*)-4-{[4-(1-benzofuran-2-yl)benzoyl]amino}-3-(methoxymethoxy)butanoate (68a)

To a stirred solution of **66a** (200 mg, 0.566 mmol) in CH_2Cl_2 (1 mL) were added *N*,*N*-diisopropylethylamine (1 mL) and methoxymethyl chloride (220 μL, 2.83 mmol) at 0 °C. After being stirred at room temperature overnight, the reaction mixture was poured into 0.5 N HCl and extracted with EtOAc. The organic layer was washed with water, 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, 1:1, as an eluent. Evaporation of the product-rich fractions provided 175 mg (78%) of **68a** as a white powder: TLC R_f = 0.38 (EtOAc–*n*-hexane, 1:1); ^{1}H NMR (200 MHz, DMSO-d_6) δ 8.65 (t, J = 5.4 Hz, 1H), 8.02 (d, J = 9.2 Hz, 2H), 7.97 (d, J = 9.2 Hz, 2H), 7.71–7.63 (m, 2H), 7.57 (br s, 1H), 7.40–7.24 (m, 2H), 4.65 (d, J = 7.0 Hz, 1H), 4.59 (d, J = 7.0 Hz, 1H), 4.20–4.08 (m, 1H), 3.58 (s, 3H), 3.52–3.34 (m, 2H), 2.65 (dd, J = 15.8, 4.4 Hz, 1H), 2.54–2.43 (m, 1H).

4.60. Methyl (3*R*)-4-{[4-(1-benzofuran-2-yl)benzoyl]amino}-3-(methoxymethoxy)butanoate (68b)

The title compound was obtained as a white powder in 75% yield from **66b** according to the same procedures as described for the preparation of **68a**: TLC R_f = 0.38 (EtOAc–*n*-hexane, 1:1); ^{1}H NMR (200 MHz, DMSO-d_6) δ 8.65 (t, J = 5.4 Hz, 1H), 8.02 (d, J = 9.2 Hz, 2H), 7.97 (d, J = 9.2 Hz, 2H), 7.71–7.63 (m, 2H), 7.57 (br s, 1H), 7.40–7.24 (m, 2H), 4.65 (d, J = 7.0 Hz, 1H), 4.59 (d, J = 7.0 Hz, 1H), 4.20–4.08 (m, 1H), 3.58 (s, 3H), 3.52–3.34 (m, 2H), 2.65 (dd, J = 15.8, 4.4 Hz, 1H), 2.54–2.43 (m, 1H).

4.61. (3*S*)-4-{[4-(1-Benzofuran-2-yl)benzoyl]amino}-3-(methoxymethoxy)butanoic acid (69a)

The title compound was obtained quantitatively from **68a** as a pale yellow powder according to the same procedures as described for the preparation of **29**: TLC R_f = 0.21 ($CHCl_3$–MeOH, 10:1); ^{1}H NMR (200 MHz, DMSO-d_6) δ 8.64 (t, J = 5.6 Hz, 1H), 8.00 (s, 4H), 7.71–7.63 (m, 2H), 7.57 (br s, 1H), 7.39–7.23 (m, 2H), 4.64 (s, 2H), 4.08 (m, 1H), 3.44 (m, 2H), 3.24 (s, 3H), 2.22 (m, 2H).

4.62. (3*R*)-4-{[4-(1-benzofuran-2-yl)benzoyl]amino}-3-(methoxymethoxy)butanoic acid (69b)

The title compound was obtained quantitatively from **68b** as a pale yellow powder according to the same procedures as described for the preparation of **29**: TLC R_f = 0.21 ($CHCl_3$–MeOH, 10:1); ^{1}H NMR (200 MHz, DMSO-d_6) δ 8.64 (t, J = 5.6 Hz, 1H), 8.00 (s, 4H), 7.71–7.63 (m, 2H), 7.57 (br s, 1H), 7.39–7.23 (m, 2H), 4.64 (s, 2H), 4.08 (m, 1H), 3.44 (m, 2H), 3.24 (s, 3H), 2.22 (m, 2H).

4.63. 4-(1-Benzofuran-2-yl)-*N*-[(2*S*)-4-(hydroxyamino)-2-(methoxymethoxy)-4-oxobutyl]benzamide (14)

The title compound was obtained as a white powder in 27% yield from **69a** according to the same

procedures as described for the preparation of **2**: TLC $R_f = 0.19$ ($CHCl_3$–MeOH, 10:1); MS (MALDI, pos.) *m/z* 421 $(M+Na)^+$, 399 $(M+H)^+$; IR (KBr) 1638, 1539, 1497, 1450, 1306, 1151, 1099, 1031 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ 10.47 (br s, 1H), 8.80 (br s, 1H), 8.64 (t, $J = 5.8$ Hz, 1H), 8.02 (d, $J = 9.2$ Hz, 2H), 7.97 (d, $J = 9.2$ Hz, 2H), 7.71–7.63 (m, 2H), 7.57 (br s, 1H), 7.40–7.24 (m, 2H), 4.60 (s, 2H), 4.16–4.05 (m, 1H), 3.41 (t, $J = 5.8$ Hz., 2H), 3.21 (s, 3H), 2.22–2.18 (m, 2H); HRMS (FAB) calcd for $C_{21}H_{23}N_2O_6$: 399.1556. Found: 399.1556.

4.64. 4-(1-Benzofuran-2-yl)-*N*-[(2*R*)-4-(hydroxyamino)-2-(methoxymethoxy)-4-oxobutyl]benzamide (15)

The title compound was obtained as a white powder in 19% yield from **69b** according to the same procedures as described for the preparation of **2**: TLC $R_f = 0.19$ ($CHCl_3$–MeOH, 10:1); MS (MALDI, pos.) *m/z* 421 $(M+Na)^+$, 399 $(M+H)^+$; IR (KBr) 1638, 1536, 1496, 1450, 1297, 1149, 1113, 1027 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ 10.46 (br s, 1H), 8.77 (br s, 1H), 8.64 (t, $J = 5.8$ Hz, 1H), 8.02 (d, $J = 9.2$ Hz, 2H), 7.97 (d, $J = 9.2$ Hz, 2H), 7.71–7.63 (m, 2H), 7.58 (br s, 1H), 7.40–7.24 (m, 2H), 4.60 (s, 2H), 4.16–4.05 (m, 1H), 3.42 (t, $J = 5.8$ Hz, 2H), 3.22 (s, 3H), 2.22–2.19 (m, 2H); HRMS (FAB) calcd for $C_{21}H_{23}N_2O_6$: 399.1556. Found: 399.1555.

4.65. (2*S*)-2-{[4-(1-Benzofuran-2-yl)benzoyl]amino}-5-methoxy-5-oxopentanoic acid (71)

To a stirred solution of 4-(1-benzofuran-2-yl)benzoyl chloride (**43**) (5.00 g, 19.5 mmol) in THF (30 mL) were added γ-methyl L-glutamate (**70**) (3.46 g, 21.5 mmol), $NaHCO_3$ powder (5.00 g, 58.5 mmol), and water (20 mL). After being stirred at room temperature overnight, the reaction 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 the volatiles in vacuo provided a solid, which was washed with $CHCl_3$–*n*-hexane to give 6.07 g (82%) of **71** as a pale yellow powder: TLC $R_f = 0.60$ ($CHCl_3$–MeOH–AcOH, 18:2:1); MS (MALDI, pos.) *m/z* 404 $(M+Na)^+$, 382 $(M+H)^+$; ^1H NMR (200 MHz, DMSO-d_6) δ 12.74 (br s, 1H), 8.74 (d, $J = 7.8$ Hz, 1H), 8.06–8.01 (m, 4H), 7.71–7.58 (m, 3H), 7.42–7.26 (m, 2H), 4.51–4.38 (m, 1H), 3.60 (s, 3H), 2.51–2.44 (m,2H), 2.26–1.96 (m, 2H).

4.66. Methyl (4*S*)-4-{[4-(1-benzofuran-2-yl)benzoyl]amino}-5-hydroxypentanoate (72)

The title compound was obtained as a white powder in 34% yield from **71** according to the same procedures as described for the preparation of **39a**: TLC $R_f = 0.47$ (EtOAc–*n*-hexane, 7:3); ^1H NMR (200 MHz, DMSO-d_6) δ 8.14 (d, $J = 8.4$ Hz, 1H), 7.99 (s, 4H), 7.72–7.60 (m, 2H), 7.55 (d, $J = 0.8$ Hz, 1H), 7.40–7.24 (m, 2H), 4.75 (t, $J = 5.8$ Hz, 1H), 4.10–3.90 (m, 1H), 3.56 (s, 3H), 3.55–3.39 (m, 2H), 2.36 (t, $J = 7.4$ Hz, 2H), 2.06–1.62 (m, 2H).

4.67. (4*S*)-4-{[4-(1-Benzofuran-2-yl)benzoyl]amino}-5-hydroxypentanoic acid (73)

The title compound was obtained as a pale pink powder in 89% yield from **72** according to the same procedures as described for the preparation of **29**: TLC $R_f = 0.33$ ($CHCl_3$–MeOH–AcOH, 190:10:1); ^1H NMR (200 MHz, DMSO-d_6) δ 12.10–11.90 (br, 1H), 8.14 (d, $J = 8.8$ Hz, 1H), 8.00 (s, 4H), 7.73–7.60 (m, 2H), 7.55 (s, 1H), 7.41–7.22 (m, 2H), 4.58 (s, 2H), 4.80–4.64 (m, 1H), 4.10–3.90 (m, 1H), 3.54–3.35 (m, 2H), 2.28 (t, $J = 6.8$ Hz, 2H), 2.02–1.60 (m, 2H).

4.68. 4-(1-Benzofuran-2-yl)-*N*-[(1*S*)-4-(hydroxyamino)-1-(hydroxymethyl)-4-oxobutyl]benzamide (16)

The title compound was obtained as a white powder in 32% yield from **73** according to the same procedures as described for the preparation of **2**: TLC $R_f = 0.22$ ($CHCl_3$–MeOH, 9:1); MS (FAB, pos.) *m/z* 369 $(M+H)^+$; IR (KBr) 3256, 1634, 1542, 1497, 1450, 1350, 1172, 1110, 1033, 979 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ 10.36 (d, $J = 1.5$ Hz, 1H), 8.67 (d, $J = 1.5$ Hz, 1H), 8.19 (d, $J = 8.4$ Hz, 1H), 8.00 (s, 4H), 7.73–7.61 (m, 2H), 7.57 (d, $J = 0.8$ Hz, 1H), 7.42–7.23 (m, 2H), 4.73 (t, $J = 5.8$ Hz, 1H), 4.08–3.85 (m, 1H), 3.58–3.38 (m, 2H), 2.12–1.60 (m, 4H); optical rotation $[\alpha]_D^{30}$ −52.52 (*c* 0.50, DMF); HRMS (FAB) calcd for $C_{20}H_{21}N_2O_5$: 369.1450. Found: 369.1447.

4.69. Methyl (4*S*)-4-{[4-(1-benzofuran-2-yl)benzoyl]amino}-5-(methoxymethoxy)pentanoate (74)

The title compound was obtained quantitatively from **72** as a beige powder according to the same procedures as described for the preparation of **68a**: TLC $R_f = 0.65$ (EtOAc–*n*-hexane, 7:3); ^1H NMR (200 MHz, $CDCl_3$) δ 7.94 (d, $J = 8.8$ Hz, 2H), 7.87 (d, $J = 8.8$ Hz, 2H), 7.64–7.50 (m, 2H), 7.38–7.21 (m, 2H), 7.13 (d, $J = 0.8$ Hz, 1H), 6.78 (d, $J = 7.8$ Hz, 1H), 4.67 (s, 2H), 4.46–4.28 (m, 1H), 3.78 (dd, $J = 10.2$, 3.4 Hz, 1H), 3.65 (dd, $J = 10.2$, 4.4 Hz, 1H), 3.64 (s, 3H), 3.39 (s, 3H), 2.62–2.38 (m, 2H), 2.16–2.00 (m, 2H).

4.70. (4*S*)-4-{[4-(1-Benzofuran-2-yl)benzoyl]amino}-5-(methoxymethoxy)pentanoic acid (75)

The title compound was obtained quantitatively from **74** as a white powder according to the same procedures as described for the preparation of **29**: TLC $R_f = 0.13$ (EtOAc–*n*-hexane, 7:3); ^1H NMR (200 MHz, DMSO-d_6) δ 12.02 (s, 1H), 8.30 (d, $J = 8.2$ Hz, 1H), 8.00 (s, 4H), 7.73–7.60 (m, 2H), 7.56 (s, 1H), 7.41–7.22 (m, 2H), 4.58 (s, 2H), 4.26–4.08 (m, 1H), 3.62–3.42 (m, 2H), 3.32 (s, 3H), 3.26 (s, 3H), 2.29 (t, $J = 6.8$ Hz, 2H), 2.02–1.60 (m, 2H).

4.71. 4-(1-Benzofuran-2-yl)-*N*-{(1*S*)-4-(hydroxyamino)-1-[(methoxymethoxy)methyl]-4-oxobutyl}benzamide (17)

The title compound was obtained as a white powder in 69% yield from **75** according to the same procedures as described for the preparation of **2**: TLC $R_f = 0.47$ ($CHCl_3$–MeOH, 9:1); MS (APCI, neg. 40 V) *m/z* 411 $(M-H)^-$; IR (KBr) 1634, 1539, 1497, 1450, 1308,

1150, 1111, 1041 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6) δ 10.37 (s, 1H), 8.34 (d, J = 8.4 Hz, 1H), 8.00 (s, 4H), 7.73–7.61 (m, 2H), 7.56 (s, 1H), 7.41–7.24 (m, 2H), 4.58 (s, 2H), 4.23–4.04 (m, 1H), 3.62–3.44 (m, 2H), 3.26 (s, 3H), 2.12–1.62 (m, 4H); optical rotation $[\alpha]_D^{30}$ −40.52 (c 0.575, DMF); HRMS (FAB) calcd for $C_{22}H_{25}N_2O_6$: 413.1713. Found: 413.1712.

4.72. (2*S*)-2-[(4-Iodobenzoyl)amino]-5-methoxy-5-oxopentanoic acid (77a)

To a stirred mixture of **70** (25.0 g, 155 mmol), $NaHCO_3$ powder (33.0 g, 388 mmol) in Et_2O–H_2O (1:1, 400 mL) was added dropwise 4-iodobenzoyl chloride (43.4 g, 163 mmol) at 0 °C. After being stirred at room temperature overnight, the reaction mixture was acidified with 2 N HCl and extracted with EtOAc. The organic layer was washed with water, brine, and dried over $MgSO_4$. Removal of the volatiles in vacuo provided a crude solid, which was washed with toluene to give 58.1 g (96%) of **77a** as a white powder: TLC R_f = 0.30 ($CHCl_3$–MeOH, 8:2); ^1H NMR (300 MHz, DMSO-d_6) δ 8.67 (d, J = 7.8 Hz, 1H), 7.87–7.84 (m, 2H), 7.67–7.63 (m, 2H), 4.40–4.32 (m, 1H), 3.65 (s, 3H), 2.44–2.39 (m, 2H), 2.18–1.89 (m, 2H).

4.73. (2*R*)-2-[(4-Iodobenzoyl)amino]-5-methoxy-5-oxopentanoic acid (77b)

The title compound was obtained as a white powder in 66% yield from **76** according to the same procedures as described for the preparation of **77a**: TLC R_f = 0.30 ($CHCl_3$–MeOH, 8:2); ^1H NMR (300 MHz, DMSO-d_6) δ 8.67 (d, J = 7.8 Hz, 1H), 7.87–7.84 (m, 2H), 7.67–7.63 (m, 2H), 4.40–4.32 (m, 1H), 3.65 (s, 3H), 2.44–2.39 (m, 2H), 2.18–1.89 (m, 2H).

4.74. Methyl (4*S*)-5-hydroxy-4-[(4-iodobenzoyl)amino]pentanoate (78a)

To a stirred solution of **77a** (58.0 g, 148 mmol) in THF (150 mL) were added *N*-hydroxysuccinimide (19.6 g, 170 mmol) and 1,3-dicyclohexylcarbodiimide (35.1 g, 170 mmol) at 0 °C. After stirring at 0 °C for 5 h, the resulting insoluble substance was removed by filtration. To the stirred filtrate were added sodium borohydride (5.60 g, 148 mmol) at 0 °C and then water (50 mL) in 30 min. After being stirred at 0 °C for 3 h, the reaction mixture was quenched with 2 N HCl and extracted with EtOAc. The organic layer was washed with saturated $NaHCO_3$, brine, and dried over $MgSO_4$. Removal of the volatiles in vacuo provided an oily solid, which was washed with toluene–EtOAc, 3:1, to give 39.3 g (71%) of **78a** as a white powder: TLC R_f = 0.36 (toluene–EtOAc, 1:1); ^1H NMR (200 MHz, $CDCl_3$) δ 7.81–7.74 (m, 2H), 7.55–7.48 (m, 2H), 6.91 (d, J = 7.0 Hz, 1H), 4.21–4.05 (m, 1H), 3.75–3.70 (m, 2H), 3.65 (s, 3H), 2.88 (t, J = 5.0 Hz, 1H), 2.53–2.45 (m, 2H), 2.04–1.94 (m, 2H).

4.75. Methyl (4*R*)-5-hydroxy-4-[(4-iodobenzoyl)amino]pentanoate (78b)

The title compound was obtained as a white powder in 81% yield from **77b** according to the same procedures as described for the preparation of **78a**. Purification was performed by silica gel chromatography with EtOAc–toluene, 4:6, as an eluent: TLC R_f = 0.36 (toluene–EtOAc, 1:1); ^1H NMR (200 MHz, $CDCl_3$) δ 7.81–7.74 (m, 2H), 7.55–7.48 (m, 2H), 6.91 (d, J = 7.0 Hz, 1H), 4.21–4.05 (m, 1H), 3.75–3.70 (m, 2H), 3.65 (s, 3H), 2.88 (t, J = 5.0 Hz, 1H), 2.53–2.45 (m, 2H), 2.04–1.94 (m, 2H).

4.76. Methyl (4*S*)-5-hydroxy-4-{[4-(3-methoxy-1-propynyl)benzoyl]amino}pentanoate (79a)

To a stirred solution of **78a** (3.00 g, 7.96 mmol) in DMF (20 mL) and triethylamine (20 mL) were added 3-methoxy-1-propyne (2.00 mL, 23.9 mmol), tetrakis(triphenylphosphine)palladium(0) (370 mg, 0.318 mmol), and copper(I) iodide (60 mg, 0.318 mmol). After being stirred at room temperature for 5 h, the reaction mixture was diluted with saturated NH_4Cl and extracted with EtOAc. The organic layer was washed with 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–$CHCl_3$, 1:30, as an eluent. Evaporation of the product-rich fractions provided 1.82 g (72%) of **79a** as a pale yellow powder: TLC R_f = 0.39 ($CHCl_3$–MeOH, 9:1); MS (MALDI, pos.) m/z 358 $(M+K)^+$, 342 $(M+Na)^+$, 320 $(M+H)^+$; ^1H NMR (200 MHz, $CDCl_3$) δ 7.76 (d, J = 8.2 Hz, 2H), 7.51 (d, J = 8.2 Hz, 2H), 6.88 (d, J = 8.0 Hz, 1H), 4.34 (s, 2H), 4.24–4.08 (m, 1H), 3.78–3.70 (m, 2H), 3.66 (s, 3H), 3.46 (s, 3H), 2.91 (t, J = 5.7 Hz, 1H), 2.55 (m, 2H), 2.01 (q, J = 6.9 Hz, 2H).

4.77. Methyl (4*R*)-5-hydroxy-4-{[4-(3-methoxy-1-propynyl)benzoyl]amino}pentanoate (79b)

To a stirred solution of **78b** (1.50 g, 4.59 mmol) in DMF (9 mL) and triethylamine (4.5 mL) were added 3-methoxy-1-propyne (482 mg, 6.89 mmol) and dichlorobis(triphenylphosphine)palladium(II) (161 mg, 0.23 mmol). After being stirred at room temperature for 3 h, the reaction mixture was diluted with 1 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 an oily residue, which was purified by silica gel chromatography with toluene–EtOAc, 3:7, as an eluent. Evaporation of the product-rich fractions provided 1.04 g (71%) of **79b** as an off-white powder: TLC R_f = 0.41 (toluene–EtOAc, 9:1); ^1H NMR (200 MHz, $CDCl_3$) δ 7.76 (d, J = 8.4 Hz, 2H), 7.50 (d, J = 8.4 Hz, 2H), 6.86 (d, J = 8.0 Hz, 1H), 4.23 (s, 2H), 4.19–4.09 (m, 1H), 3.73–3.71 (m, 2H), 3.65 (s, 3H), 3.46 (s, 3H), 2.54–2.45 (m, 2H), 2.05–1.95 (m, 2H).

4.78. (4*S*)-5-Hydroxy-4-{[4-(3-methoxy-1-propynyl)benzoyl]amino}pentanoic acid (80a)

The title compound was obtained as a pale yellow powder in 77% yield from **79a** according to the same procedures as described for the preparation of **29**: MS (MALDI, pos.) m/z 344 $(M+K)^+$, 328 $(M+Na)^+$, 306 $(M+H)^+$; ^1H NMR (200 MHz, DMSO-d_6) δ 12.00 (s, 1H), 8.12 (d, J = 8.4 Hz, 1H), 7.87 (d, J = 8.3 Hz, 2H),

7.54 (d, J = 8.3 Hz, 2H), 4.73 (t, J = 5.5 Hz, 1H), 4.35 (s, 2H), 4.04–3.87 (m, 1H), 3.55–3.33 (m, 5H), 2.25 (t, J = 7.3 Hz, 2H), 1.99–1.58 (m, 2H).

4.79. (4*R*)-5-Hydroxy-4-{[4-(3-methoxy-1-propynyl)benzoyl]amino}pentanoic acid (80b)

The title compound was obtained as an off-white powder in 99% yield from **79b** according to the same procedures as described for the preparation of **29**: TLC R_f = 0.59 ($CHCl_3$–MeOH–AcOH–H_2O, 85:15:1:1); ^1H NMR (200 MHz, DMSO-d_6) δ 11.98 (br s, 1H), 8.12 (d, J = 8.4 Hz, 1H), 7.85 (d, J = 8.2 Hz, 2H), 7.53 (d, J = 8.2 Hz, 2H), 4.34 (s, 2H), 4.05–3.83 (m, 1H), 3.50–3.20 (m, 2H), 3.28 (s, 2H), 2.23–2.19 (m, 2H), 1.89–1.50 (m, 2H).

4.80. *N*-[(1*S*)-4-(Hydroxyamino)-1-(hydroxymethyl)-4-oxobutyl]-4-(3-methoxy- 1-propynyl)benzamide (18)

The title compound was obtained as a pale yellow powder in 30% yield from **80a** according to the same procedures as described for the preparation of **2**: TLC R_f = 0.36 ($CHCl_3$–MeOH, 4:1); MS (APCI, pos. 20 V) m/z 321 $(M+H)^+$; IR (KBr) 1635, 1547, 1502, 1451, 1314, 1280, 1187, 1095, 1047 cm^{-1}; ^1H NMR (200MHz, DMSO-d_6) δ 10.35 (s, 1H), 10.18 (s, 1H), 8.18 (d, J = 8.4 Hz, 1H), 7.88 (d, J = 8.4 Hz, 2H), 7.55 (d, J = 8.4 Hz, 2H), 4.53 (s, 2H), 4.02–3.84 (m, 1H), 3.73–3.34 (m, 2H), 3.35 (s, 3H), 2.07–1.59 (m, 4H); optical rotation $[\alpha]_D^{30}$ −29.4 (c 0.31, DMF); HRMS (FAB) calcd for $C_{16}H_{21}N_2O_5$: 321.1450. Found: 321.1446.

4.81. *N*-[(1*R*)-4-(Hydroxyamino)-1-(hydroxymethyl)-4-oxobutyl]-4-(3-methoxy-1-propynyl)benzamide (19)

The title compound was obtained as an ivory powder in 13% yield from **80b** according to the same procedures as described for the preparation of **2**: TLC R_f = 0.28 ($CHCl_3$–MeOH–AcOH–H_2O, 85:15:1:1); MS (MALDI, pos.) m/z 343 $(M+Na)^+$, 321 $(M+H)^+$; IR (KBr) 1630, 1548, 1503, 1446, 1352, 1186, 1102, 1048, 972 cm^{-1}; ^1H NMR (200 MHz, CD_3OD) δ 7.83 (d, J = 8.4 Hz, 2H), 7.51 (d, J = 8.4 Hz, 2H), 4.34 (s, 2H), 4.03–4.15 (m, 1H), 3.62 (d, J = 5.6 Hz, 2H), 3.43 (s, 3H), 2.19 (t, J = 7.4 Hz, 2H), 1.77–2.10 (m, 2H); optical rotation $[\alpha]_D^{30}$ +15.2 (c 0.11, DMF); HRMS (FAB) calcd for $C_{16}H_{21}N_2O_5$: 321.1450. Found: 321.1452.

4.82. Methyl (4*S*)-5-(methoxymethoxy)-4-{[4-(3-methoxy-1-propynyl)benzoyl]amino}pentanoate (81a)

The title compound was obtained quantitatively from **79a** as a pale yellow solid according to the same procedures as described for the preparation of **68a**: TLC R_f = 0.74 ($CHCl_3$–MeOH, 9:1); MS (MALDI, pos.) m/z 402 $(M+K)^+$, 386 $(M+Na)^+$, 364 $(M+H)^+$; ^1H NMR (200 MHz, $CDCl_3$) δ 7.75 (d, J = 8.4 Hz, 2H), 7.51 (d, J = 8.4 Hz, 2H), 6.72 (d, J = 8.8 Hz, 1H), 4.65 (s, 2H), 4.41–4.25 (m, 3H), 3.79–3.58 (m, 5H), 3.46 (s, 3H), 3.38 (s, 3H), 2.60–2.35 (m, 2H), 2.15–1.94 (m, 2H).

4.83. Methyl (4*R*)-5-(Methoxymethoxy)-4-{[4-(3-methoxy-1-propynyl)benzoyl]amino}pentanoate (81b)

The title compound was obtained quantitatively from **79b** as an off-white powder according to the same procedures as described for the preparation of **68a**: TLC R_f = 0.57 (toluene–EtOAc, 1:1); ^1H NMR (200 MHz, $CDCl_3$) δ 7.74 (d, J = 8.4 Hz, 2H), 7.52 (d, J = 8.4 Hz, 2H), 6.70 (d, J = 8.8 Hz, 1H), 4.64 (s, 2H), 4.33 (s, 2H), 4.40–4.25 (m, 1H), 3.75 (dd, J = 10.4, 3.4 Hz, 1H), 3.61 (dd, J = 10.4, 3.4 Hz, 1H), 3.62 (s, 3H), 3.46 (s, 3H), 3.37 (s, 3H), 2.58–2.38 (m, 2H), 2.12–1.93 (m, 2H).

4.84. (4*S*)-5-(Methoxymethoxy)-4-{[4-(3-methoxy-1-propynyl)benzoyl]amino}pentanoic acid (82a)

The title compound was obtained as an off-white powder in 77% yield from **81a** according to the same procedures as described for the preparation of **29**: MS (MALDI, pos.) m/z 372 $(M+Na)^+$, 350 $(M+H)^+$; ^1H NMR (200 MHz, DMSO-d_6) δ 8.28 (d, J = 8.8 Hz, 1H), 7.86 (d, J = 8.5 Hz, 2H), 7.55 (d, J = 8.5 Hz, 2H), 4.57 (s, 2H), 4.35 (s, 2H), 4.22–4.04 (m, 1H), 3.55–3.45 (m, 2H), 3.35 (s, 3H), 3.24 (s, 3H), 2.27 (t, J = 7.5 Hz, 2H), 1.97–1.64 (m, 2H).

4.85. (4*R*)-5-(Methoxymethoxy)-4-{[4-(3-methoxy-1-propynyl)benzoyl]amino}pentanoic acid (82b)

The title compound was obtained as an off-white powder in 98% yield from **81b** according to the same procedures as described for the preparation of **29**: TLC R_f = 0.47 ($CHCl_3$–MeOH–AcOH–H_2O, 100:10:1:1); ^1H NMR (200 MHz, DMSO-d_6) δ 8.28 (d, J = 8.8 Hz, 1H), 7.86 (d, J = 8.5 Hz, 2H), 7.55 (d, J = 8.5 Hz, 2H), 4.57 (s, 2H), 4.35 (s, 2H), 4.22–4.04 (m, 1H), 3.55–3.45 (m, 2H), 3.35 (s, 3H), 3.24 (s, 3H), 2.27 (t, J = 7.5 Hz, 2H), 1.97–1.64 (m, 2H).

4.86. *N*-{(1*S*)-4-(hydroxyamino)-1-[(methoxymethoxy)methyl]-4-oxobutyl}-4-(3-methoxy-1-propynyl)-benzamide (20)

The title compound was obtained as a yellow oil in 94% yield from **82a** according to the same procedures as described for the preparation of **2**: TLC R_f = 0.22 ($CHCl_3$–MeOH, 9:1); MS (MALDI, pos.) m/z 387 $(M+Na)^+$, 365 $(M+H)^+$; IR (neat) 1643, 1544, 1501, 1452, 1357, 1150, 1101, 1040 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6):δ 10.35 (s, 1H), 8.32 (d, J = 8.6 Hz, 1H), 7.87 (d, J = 8.4 Hz, 2H), 7.55 (d, J = 8.4 Hz, 2H), 4.57 (s, 2H), 4.36 (s, 2H), 4.20–4.01 (m, 1H), 3.55–3.44 (m, 2H), 3.35 (s, 3H), 3.24 (s, 3H), 2.09–1.64 (m, 4H); optical rotation $[\alpha]_D^{30}$ −25.6 (c 0.25, DMF); HRMS (FAB) calcd for $C_{18}H_{25}N_2O_6$: 365.1713. Found: 365.1704.

4.87. *N*-{(1*R*)-4-(Hydroxyamino)-1-[(methoxymethoxy)methyl]-4-oxobutyl}-4-(3-methoxy-1-propynyl)benzamide (21)

The title compound was obtained as a yellow gum in 72% yield from **82b** according to the same procedures as described for the preparation of **2**: TLC R_f = 0.25

($CHCl_3$–MeOH–AcOH–H_2O, 100:10:1:1); MS (MALDI, pos.) *m/z* 387 $(M + Na)^+$, 365 $(M+H)^+$; IR (KBr) 1640, 1542, 1500, 1451, 1356, 1213, 1186, 1150, 1100, 1038, 965 cm^{-1}; ^1H NMR (200 MHz, CD_3OD) δ 8.32 (d, $J = 8.8$ Hz, 1H), 7.82 (d, $J = 8.8$ Hz, 2H), 7.52 (d, $J = 8.8$ Hz, 2H), 4.62 (s, 2H), 4.34 (s, 2H), 4.16–4.31 (m, 1H), 3.62 (d, $J = 5.6$ Hz, 2H), 3.43 (3H, s), 3.33 (3H, s), 2.20 (t, $J = 7.0$ Hz, 2H), 1.17–2.11 (m, 2H); optical rotation $[\alpha]_D^{30}$ +5.60 (*c* 0.23, MeOH); HRMS (FAB) calcd for $C_{18}H_{25}N_2O_6$: 365.1713. Found: 365.1722.

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

5.1. 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 U/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.

5.2. MMP-2 and MMP-9 assays[11]

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. Enzyme solution (50 μL) was added and the reaction was performed at 37 °C for 10 min. Its fluorescence was measured by f_{max}. Excitation and emission wavelengths were 320 and 390 nm, respectively.

5.3. MMP-3 assay[12]

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. Enzyme solution (50 μL) was added and the reaction was performed at 37 °C for 10 min. Its fluorescence was measured by f_{max}. Excitation and emission wavelengths were 320 and 390 nm, respectively.

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