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Novel 6-Hydroxy-3-morpholinones as Cornea Permeable Calpain Inhibitors

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Abstract—A novel series of 6-hydroxy-3-morpholinones, in which the functional aldehyde and the hydroxy group of P_2 site form a cyclic hemiacetal, was identified as calpain inhibitors. The placement of isobutyl group at the 2-position of the 3-morpholinone was the most effective modification for inhibiting μ- and *m*-calpains. Substitutions of benzyl at the 5-position in the *S*-configuration had virtually no effect on inhibitory activity. Several compounds showed appreciable selectivity for calpains over cathepsin B. NMR experiments demonstrated that the representative 6-hydroxy-3-morpholinone **10a** (SNJ-1757) was more stable to nucleophilic attack than the corresponding aldehyde inhibitor **24**. Furthermore, 6-hydroxy-3-morpholinone **10a** proved to have better corneal permeability than aldehyde inhibitor **24** in an in vitro experiment.

Introduction

Calpains are cytosolic calcium-dependent cysteine endoproteases and the family of these enzymes has grown rapidly in recent years.[1] Two major calpains, μ-calpain and *m*-calpain, are widely distributed in mammalian cells, and have been implicated in a variety of biological processes, and in numerous diseases such as stroke, Alzheimer's disease, spinal cord injury, cardiac ischaemia, muscular dystrophy and cataract.[2] One cause of opacification in cataract has been considered to be calpain-induced proteolysis of crystallins in the lens, as this interrupts the normal interaction between lens proteins, and leads to aggregation.[3] The transcorneal permeability of a drug is an important factor in the ability to treat intraocular diseases like cataract by means of ophthalmic solution. Therefore, our goal in this study was to design cornea permeable calpain inhibitors for development as anticataract agents.

Peptidyl aldehydes are the classical inhibitors for cysteine proteases such as calpains, cathepsins B, K and L and caspases.[4] In region of oral drug developments, several groups have made an issue of poor absorption of the aldehyde inhibitors,[5] whereas the cause has not been solved yet. We predicted that the aldehyde group may have superfluous reaction with various biological substances or proteins through Schiff base or thiohemiacetal formation under the physiological conditions, and this may limit drug penetration across the membranes such as intestine and cornea. In fact, we have already reported that conversion of aldehyde to a cyclic hemiacetal that formed by the aldehyde group and hydroxyalkyl side residue at P_1 site improved the transcorneal permeability of a peptide-based calpain inhibitor.[6] In order to extend this study, we designed a type of new calpain inhibitors based on the following hypotheses: (1) Compound **2** (Fig. 1) with a hydroxy group instead of an amide bond between the P_3-P_2 sites of general dipeptidyl aldehyde inhibitors (**1**), might form the cyclized structure **3** by cyclic hemiacetal formation. (2) This cyclization might limit the chemical reactivity of the aldehyde group and reduce superfluous reaction under physiological conditions. (3) Linear isomer **2** might fit the enzyme pocket as peptidyl aldehyde inhibitors do, and so have inhibitory activity. (4) The hydroxy group of linear isomer **2** might act as a proton donor instead of NH of the amide bond. (5) The placement of isopropyl or isobutyl at the R′ side residue might be advantageous for calpain inhibition, by analogy with most of the synthetic peptide-based calpain inhibitors containing L-Val or L-Leu at P_2 site.[7]

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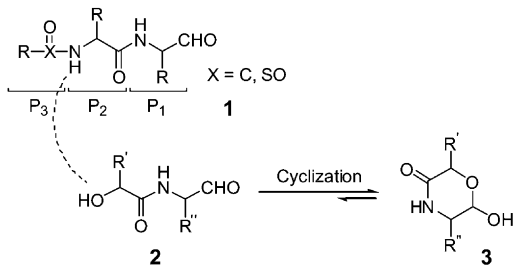


Figure 1. Strategy for synthesis of 6-hydroxy-3-morpholinone inhibitors.

In this report, we describe the synthesis, inhibitory activities and transcorneal permeability of cyclized compounds **3** as novel 6-hydroxy-3-morpholinone calpain inhibitors.

Results and Discussion

Chemistry

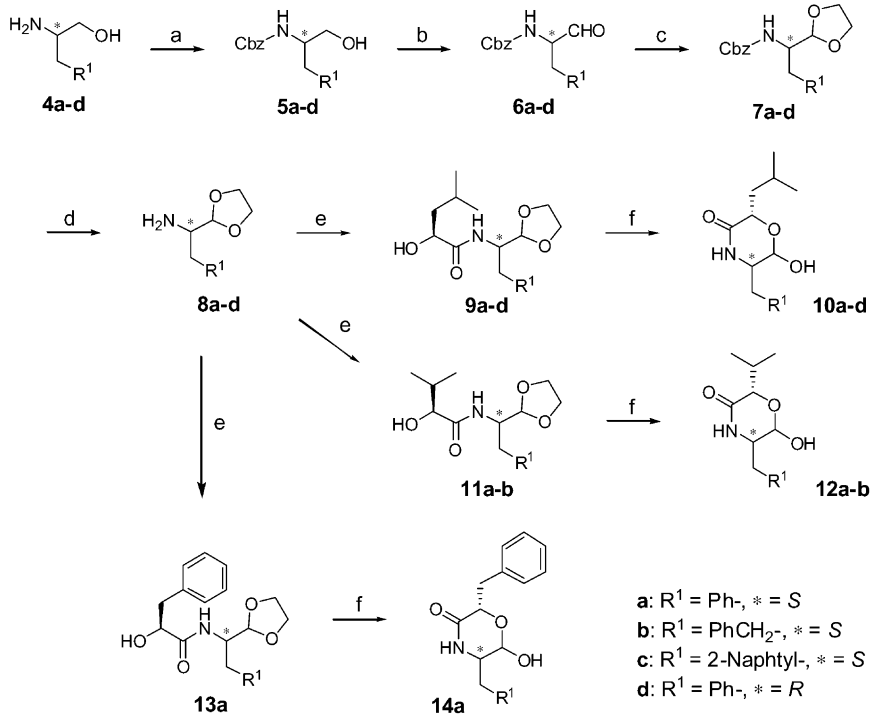
6-Hydroxy-3-morpholinones **10a–d**, **12a–b** and **14a** were synthesized as shown in Scheme 1. Treatment of amino alcohol **4** with *N*-carbobenzoxyoxy succinimide (Cbz-OSu) followed by DMSO oxidation with sulfurtrioxide/pyridine complex (SO_3/pyridine) provided aldehyde **6**. The aldehyde group of this compound was protected by the formation of acetal with ethylene glycol, and subsequent hydrogenolysis of the Cbz-protecting group provided intermediate **8**. Condensation of **8** and each α-hydroxy acetic acid in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) and 1-hydroxybenzotriazole (HOBt) followed by hydrolysis of the acetal under acidic condition led to target compounds **10a–d**, **12a–b** and **14a** which were unambiguously confirmed as cyclized structure by ^{1}H NMR analysis.

Tyrosine-based 6-hydroxy-3-morpholinones **22a–d** were synthesized as shown in Scheme 2. Treatment of L-tyrosine methyl ester with Cbz-OSu produced Cbz-tyrosine methyl ester **16a**, and subsequent *O*-alkylation with dimethyl sulfate or alkyl bromide gave rise to the *O*-alkylated Cbz-tyrosine methyl ester **16b–d**. Reduction of **16** with $NaBH_4$ followed by DMSO oxidation with SO_3/pyridine, produced aldehyde **18**. The aldehyde group of this compound was protected by the formation of acetal with ethylene glycol, and subsequent hydrogenolysis of the Cbz-protecting group provided intermediate **20**. Condensation of **20** and L-leucic acid with EDC and HOBt followed by hydrolysis of the acetal led to tyrosine-based 6-hydroxy-3-morpholinone **22**.

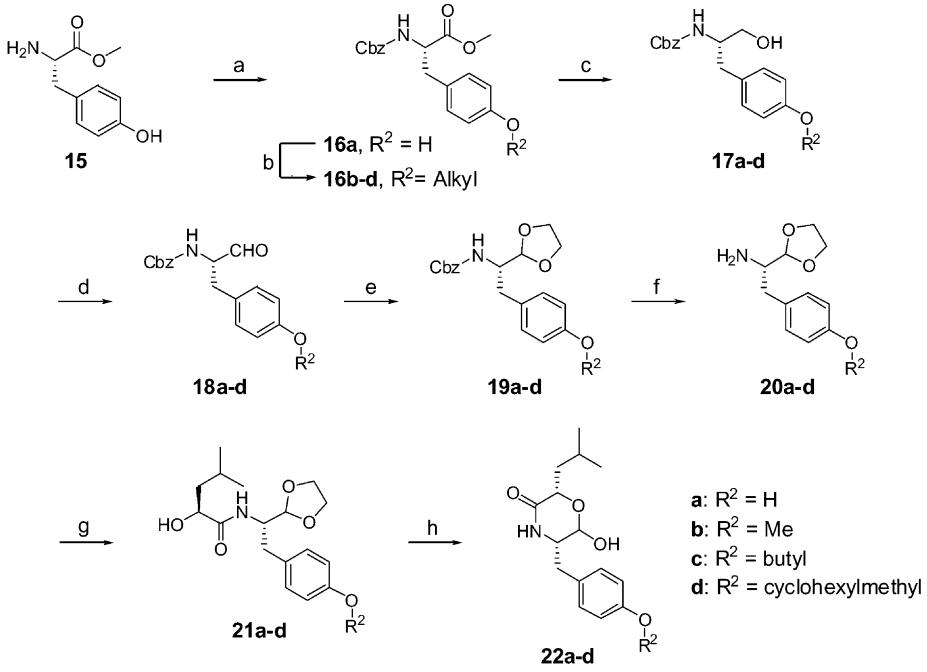
P_3-Truncated aldehyde inhibitor **24** was synthesized as shown in Scheme 3. Acylation of L-phenylalaninol (**4a**) with 4-methylpentanoic acid in the presence of EDC and HOBt followed by SO_3/pyridine oxidation produced aldehyde **24**.

Enzyme inhibition and water-solubility

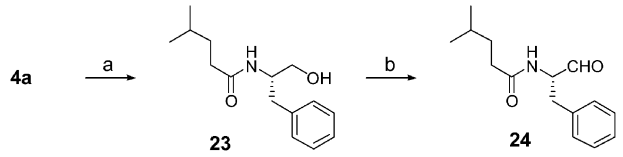
The inhibitory activities and water-solubility of the 6-hydroxy-3-morpholinones are shown in Table 1. We first investigated the influence of the 2-position side residue of 6-hydroxy-3-morpholinone, and placement of isobutyl group proved to be the most effective in inhibiting μ- and *m*-calpains (**10a** vs **12a** vs **14a**). Isopropyl compound **12a** was only 2- to 3-fold less active than isobutyl compound **10a**. Additionally, the relation



Scheme 1. Conditions: (a) Cbz-OSu, THF; (b) SO_3/pyridine, DIPEA, DMSO/CH_2Cl_2; (c) ethylene glycol, pyridinium *p*-toluenesulfonate, toluene; (d) Pd/C, EtOAc; (e) α-hydroxy acetic acids, HOBt, Et_3N, EDC, DMF/CH_2Cl_2; (f) 6 M HCl, THF.



Scheme 2. Conditions: (a) Cbz-OSu, THF; (b) $(CH_3)_2SO_4$, K_2CO_3, acetone; or alkyl bromide, K_2CO_3, DMF; (c) $NaBH_4$, LiCl, THF/EtOH; (d) SO_3/pyridine, DIPEA, DMSO/CH_2Cl_2; (e) ethylene glycol, pyridinium *p*-toluenesulfonate, toluene; (f) Pd/C, EtOAc; (g) L-leucic acid, HOBt, Et_3N, EDC, DMF/CH_2Cl_2; (h) 6 M HCl, THF.



Scheme 3. Conditions: (a) 4-methylpentanoic acid, HOBt, Et_3N, EDC, DMF/CH_2Cl_2; (b) SO_3/pyridine, DIPEA, DMSO/CH_2Cl_2.

between activities of isobutyl compound **10b** and isopropyl compound **12b** is also similar to those of **10a** and **12a**. The substitution with benzyl group (**14a**) significantly decreased inhibition (about 20-fold less active). Thus, it appears that the isobutyl group and isopropyl group are important for the inhibitory activities of the class of 6-hydroxy-3-morpholinone calpain inhibitors, and we consider that they play the same role as the side residues of L-Leu or L-Val at the P_2 site of peptide-based inhibitors. On the other hand, truncated aldehyde inhibitor **24**, in which the hydroxyl group has been removed from linear isomer of **10a**, exhibited marginally better activities against calpains than **10a**. However, a synthetic intermediate, aldehyde **6a**, was 10-fold less potent against calpains than aldehyde **24**. These results suggest that placement of branched alkyl in the vicinity of the P_2 site is important for calpain inhibition even if the aldehyde inhibitor does not contain a P_3 residue.

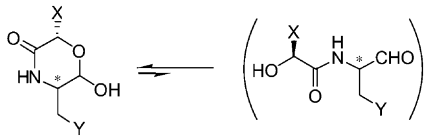
Next we explored the influence of the side residue at the 5-position. Compound **10d**, in which the chiral center formed by the benzyl substitution has been inverted, resulted in about 5-fold decrease in inhibitory activities as compared to **10a**. Varying substitution at the 5-position with *S*-configuration had virtually no effect on the inhibitory activities (**10a–c** and **22a–d**). Even substitution of 4-cyclohexylmethoxybenzyl (**22d**), the largest substitution made, had no significant effect. If we suppose that the active form is a linear isomer, the lack of influence of substitutions at the 5-position is in accord with previous reports[7] that a variety of P_1-side residues are tolerated in peptide-based calpain inhibitors and substrates. Therefore, from the SAR of 2- and 5-positions, we consider that, linear isomers which are structurally similar to known peptidyl aldehydes, bind to the enzymes and they exhibit calpain inhibition. However, to confirm that the active form is a linear isomer, the X-ray crystal structure of the enzyme–inhibitor complex should be determined.

Overall, this type of 6-hydroxy-3-morpholinone is a modest calpain inhibitor, being approximately equipotent with known peptidyl aldehyde leupeptin, and it is less potent inhibitors than peptidyl aldehyde SJA6017.[6,8] Although most of known peptidyl aldehydes such as SJA6017 and leupeptin are non-selective calpain inhibitors, 6-hydroxy-3-morpholinone inhibitors are comparatively selective over cathepsin B. Compounds **10a**, **10d** and **22b**, in particular, have good selectivity. Furthermore, compound **10a** possesses good water-solubility (2.0 mg/mL), being about 2-fold more soluble than the corresponding truncated aldehyde **24**. However, substitution of 5-positions with naphthyl (**10c**) as bulkier residue remarkably decreased the water-solubility (0.093 mg/mL).

Casein zymogram

We performed a casein zymogram analysis,[9] in order to identify the binding profile of 6-hydroxy-3-morpholinone **10a** (Fig. 2). When *m*-calpain was pre-incubated without calcium ions for 10 min, run into casein gel by

Table 1. Inhibitor activity and water-solubility



	2-Position (X)	5-Position (Y)	Chiral (∗)	IC_{50} (μM)			Water solubility[d] (mg/mL)
				μ-Calpain[a]	*m*-Calpain[b]	Cathepsin B[c]	
10a	Isobutyl	Ph–	*S*	0.70	0.93	>100	2.0
10b	Isobutyl	$PhCH_2$–	*S*	0.51	1.3	55	nd
10c	Isobutyl	2–Naphtyl–	*S*	0.49	1.2	28	0.093
10d	Isobutyl	Ph–	*R*	5.9	10	>100	nd
12a	Isopropyl	Ph–	*S*	2.3	2.1	18	1.3
12b	Isopropyl	$PhCH_2$–	*S*	3.5	5.4	15	nd
14a	Benzyl	Ph–	*S*	17	19	23	nd
22a	Isobutyl	4–(OH)–Ph–	*S*	2.5	1.9	nd	nd
22b	Isobutyl	4–(MeO)–Ph–	*S*	0.68	2.2	>100	nd
22c	Isobutyl	4–(Butoxy)–Ph–	*S*	0.52	1.2	49	nd
22d	Isobutyl	4–(cHexMeO)–Ph–	*S*	0.45	1.4	nd	nd
6a				5.5	9.6	13	nd
24				0.29	0.65	18	0.91
Leupeptin				0.62	0.59	0.027	nd
SJA6017				0.022	0.049	0.0069	0.10

[a]Human erythrocyte μ-calpain.
[b]Porcine kidney *m*-calpain.
[c]Human liver cathepsin B.
[d]Water-solubility in pH 7 buffer, at 25 °C.

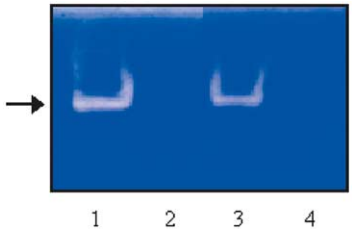


Figure 2. Casein zymogram of *m*-calpain activity. *m*-Calpain was pre-incubated: (1) without Ca^{2+}, (2) with Ca^{2+} (3) with Ca^{2+} and 100 μM **10a**, (4) with Ca^{2+} and 100 μM E64. After subsequent electrophoresis, the gel was soaked in Ca^{2+} buffer. White bands of 80 kDa (arrow) show caseinolysis by active *m*-calpain.

electrophoresis and then soaked in calcium-activating buffer, 80 kDa white band was observed resulting from caseinolysis by *m*-calpain (lane 1). In contrast, when *m*-calpain was pre-incubated with calcium ions for 10 min, caseinolysis was not observed (lane 2); during the pre-incubation, calcium ions activated the *m*-calpain, and subsequently caused its autolytic degradation. When the irreversible inhibitor E64 was present during pre-incubation, caseinolysis was not observed (lane 4); the electrophoresed enzyme did not recover the activity, because E64 had bound it irreversibly in the pre-incubation mixture. Most importantly, when compound **10a** instead of E64 was added, caseinolysis was observed (lane 3); compound **10a** evidently inhibited the *m*-calpain in the pre-incubation mixture, but then dissociated from it during electrophoresis. We conclude that 6-hydroxy-3-morpholinone **10a** is a reversible inhibitor.

NMR experiment

On the assumption that aldehyde inhibitors can bind to a variety of substances under physiological condition, the stabilities of 6-hydroxy-3-morpholinone **10a** and aldehyde **24** were studied by ^1H NMR. Semicarbazide ($H_2NNHCONH_2$) hydrochloride, which simulates biomolecules containing free amines, was used as nucleophile, and the NMR spectra of compound **10a** and **24** were determined in the presence and absence of semicarbazide in DMSO-d_6/D_2O (3:1). A singlet peak from the aldehyde proton was observed in the spectrum of aldehyde **24** in the absence of semicarbazide, but the addition of semicarbazide resulted in the formation of a hydrazone compound and abolished the aldehyde peak (Fig. 3A and B). Thus, the aldehyde inhibitor binds efficiently to the amine component, forming a Schiff base. In contrast, the spectra of 6-hydroxy-3-morpholinone **10a** in the presence and absence of semicarbazide were identical (Fig. 3C and D). Hence, the moderate protection of the aldehyde group as a result of cyclic hemiacetal formation led to increased resistance to nucleophilic attack. We therefore consider 6-hydroxy-3-morpholinone inhibitors to be superior to peptide-based aldehyde inhibitors with respect to permeability and stability under physiological conditions.

In vitro corneal drug permeability

The in vitro corneal permeability of compounds **10a** and **24** was evaluated using isolated albino rabbit corneas in a modified Ussing chamber.[10] The corneal penetration profiles are shown in Figure 4; the calculated apparent

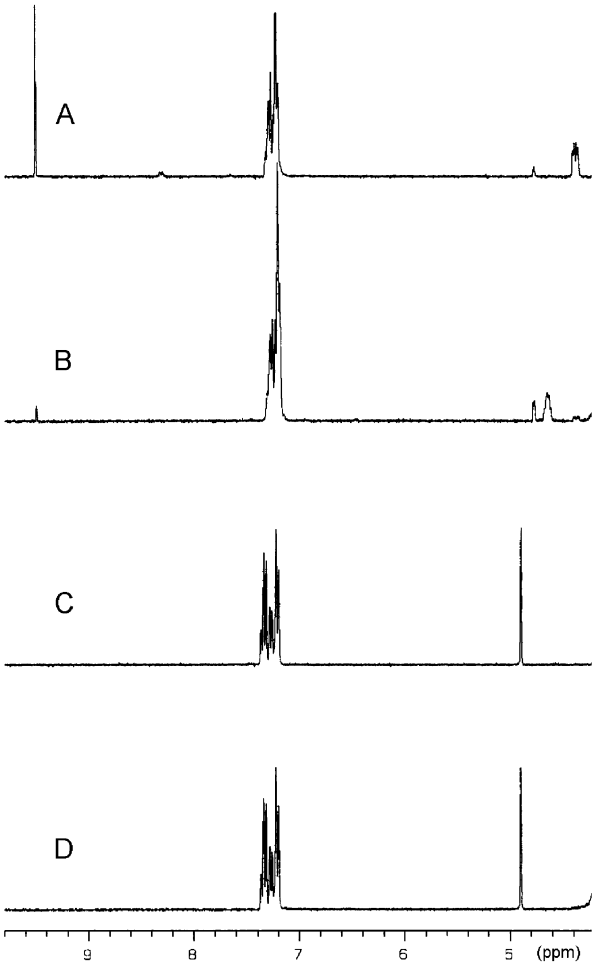


Figure 3. ^{1}H NMR (300 MHz) spectra of compounds **10a** and **24** in DMSO-d_6/D_2O (3:1); (A) 25 mM compound **24**, (B) 25 mM compound **24** with 25 mM semicarbazide, (C) 25 mM compound **10a**, (D) 25 mM compound **10a** with 25 mM semicarbazide.

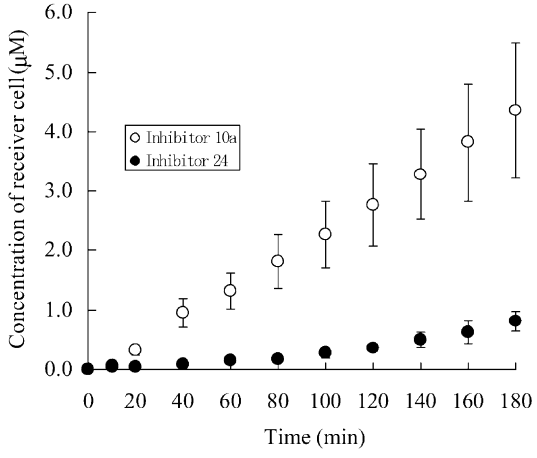


Figure 4. Profile of penetration of inhibitors **10a** and **24** through the isolated rabbit cornea. Each value represents the average of three experiments, and the vertical bars are SDs.

permeability coefficients (P_{app}) of compounds **10a** and **24** were 1.9 ± 0.51 ($\times10^{-5}$, cm/s) and 0.54 ± 0.19 ($\times10^{-5}$, cm/s), respectively. We believe that the more effective penetration of 6-hydroxy-3-morpholine **10a** is attributable to resistance of the functional aldehyde group to nucleophilic attack based on the cyclic hemiacetal formation. However, it may not only depend on this resistance but also on some intrinsic property of its structure.

Conclusions

We have described 6-hydroxy-3-morpholinones, a new class of calpain inhibitors, in which the functional aldehyde and the hydroxy group of P_2 site form a cyclic hemiacetal. Although 6-hydroxy-3-morpholinones are modest calpain inhibitors, they possess good selectivity over cathepsin B. A casein zymogram revealed that they are reversible inhibitors, and NMR showed that 6-hydroxy-3-morpholinone **10a** is more resistant to nucleophilic attack than aldehyde inhibitor **24**. Moreover, an in vitro experiment showed that compound **10a** has good corneal permeability.

Although much effort has gone into the development of peptidyl aldehyde as cysteine protease inhibitors, none has yet been launched clinically. One reason for this may be the occurrence of superfluous reaction of the aldehyde group that result in reduced drug penetration. The synthesis, in the present work, of 6-hydroxy-3-morpholinones in which the functional aldehyde is protected by cyclic hemiacetal formation, successfully generated cornea permeable calpain inhibitors. Compound **10a** is going to be employed in in vivo studies, and we hope that the present approach will contribute to the development of potent cysteine protease inhibitors.

Experimental

General

Melting points were determined on a Yanaco micro melting point apparatus without correction. ^{1}H NMR spectra were recorded on a 300 MHz Varian Gemini-2000 spectrometer. Chemical shifts are reported in parts per million, and coupling constants (J) are reported in Hertz. Elemental analyses were performed on an Elementar Vario EL analyzer. High-resolution electrospray ionization mass spectra (ESI-MS) were obtained by a Micromass LCT mass spectrometer. Matrix-assisted laser desorption ionization time-of-flight mass spectra (MALDI-TOF-MS) were obtained on a Perseptive Voyager DE mass spectrometer, and the mass numbers were corrected with a internal standard (α-cyano-4-hydroxycinamic acid) and displayed accurately. Optical rotations were measured in a Horiba SEPA-2000 model.

Typical procedure for the preparation of compounds 5a–d

***N*-(Benzyloxycarbonyl)-L-phenylalaninol (5a).** To a solution of L-phenylalaninol (**4a**) (25 g, 170 mmol) in THF (250 mL) were added Cbz-OSu (41 g, 170 mmol) and Et_3N (25 g, 250 mmol) under the ice cool condition. The mixture was stirred at room temperature for 18 h. After concentration in vacuo, the residue was dissolved in EtOAc, and the solution was washed with 1 M HCl,

saturated $NaHCO_3$ and saturated NaCl, dried over $MgSO_4$ and concentrated in vacuo. The residue was crystallized from hexane to give **5a** (41 g, 87%) as colorless crystals. Mp 90–91 °C. ^1H NMR (DMSO-d_6) δ 2.58 (dd, 1H, $J=13.8$, 9.3 Hz), 2.85 (dd, 1H, $J=13.8$, 5.5 Hz), 3.27–3.42 (m, 2H), 3.66 (m, 1H), 4.76 (t, 1H, $J=5.5$ Hz), 4.91–5.00 (m, 2H), 7.12 (d, 1H, $J=8.4$ Hz), 7.16–7.37 (m, 10H). Anal. calcd for $C_{17}H_{19}NO_3$: C, 71.56; H, 6.71; N, 4.91; found: C, 71.45; H, 6.72; N, 4.68.

***N*-(Benzyloxycarbonyl)-L-homophenylalaninol (5b).** Yield 92%. Colorless crystals. Mp 120–121 °C. ^1H NMR (DMSO-d_6) δ 1.59 (m, 1H), 1.79 (m, 1H), 2.46–2.68 (m, 2H), 3.23–3.45 (m, 3H), 4.65 (t, 1H, $J=5.4$ Hz), 5.03 (s, 2H), 7.09–7.44 (m, 11H). Anal. calcd for $C_{18}H_{21}NO_3$: C, 72.22; H, 7.07; N, 4.68; found: C, 72.51; H, 7.20; N, 4.86.

***N*-(Benzyloxycarbonyl)-3-(2-naphthyl)-L-alaninol (5c).** Yield 93%. Colorless crystals. Mp 75–77 °C. ^1H NMR (DMSO-d_6) δ 2.75 (dd, 1H, $J=14.1$, 9.0 Hz), 3.04 (dd, 1H, $J=13.7$, 5.0 Hz), 3.36–3.47 (m, 2H), 3.77 (m, 1H), 4.82 (t, 1H, $J=5.4$ Hz), 4.92 (dd, 2H, $J=18.5$, 13.1 Hz), 7.18–7.25 (m, 6H), 7.39–7.51 (m, 3H), 7.71 (s, 1H), 7.81–7.89 (m, 3H). Anal. calcd for $C_{21}H_{21}NO_3$: C, 75.20; H, 6.31; N, 4.18; found: C, 74.84; H, 6.34; N, 3.99.

***N*-(Benzyloxycarbonyl)-D-phenylalaninol (5d).** Yield 89%. Colorless crystals. Mp 76 °C. ^1H NMR (DMSO-d_6) δ 2.58 (dd, 1H, $J=13.6$, 9.0 Hz), 2.85 (dd, 1H, $J=13.6$, 5.3 Hz), 3.27–3.42 (m, 2H), 3.66 (m, 1H), 4.75 (t, 1H, $J=5.7$), 4.91–5.00 (m, 2H), 7.11 (d, 1H, $J=8.4$ Hz), 7.16–7.37 (m, 10H). Anal. calcd for $C_{17}H_{19}NO_3$: C, 71.56; H, 6.71; N, 4.91; found: C, 71.38; H, 6.63; N, 5.00.

Typical procedure for the preparation of compounds 6a–d

***N*-(Benzyloxycarbonyl)-L-phenylalaninal (6a).** Compound **5a** (40 g, 140 mmol) was dissolved in DMSO (160 mL) and CH_2Cl_2 (80 mL), and cooled under the ice cool condition. *N,N*-Diisopropylethylamine (54 g, 420 mmol) and a suspension of SO_3/pyridine (67 g, 420 mmol) in DMSO (80 mL) were added thereto. The mixture was stirred for 30 min under the same conditions. The reaction mixture was diluted with EtOAc, and the solution was washed with 1 M HCl, saturated $NaHCO_3$ and saturated NaCl, dried over $MgSO_4$ and concentrated in vacuo. The residue was crystallized from hexane/EtOAc to give **6a** (30 g, 76%) as colorless crystals. Mp 66–67 °C. ^1H NMR (DMSO-d_6) δ 2.72 (dd, 1H, $J=14.1$, 10.4 Hz), 3.15 (dd, 1H, $J=14.1$, 4.2 Hz), 4.2 (m, 1H), 4.96–5.05 (m, 2H), 7.19–7.39 (m, 10H), 7.76 (d, 1H, $J=7.8$ Hz), 9.57 (s, 1H). Anal. calcd for $C_{17}H_{17}NO_3$: C, 72.07; H, 6.05; N, 4.94; found: C, 71.69; H, 6.30; N, 4.76.

***N*-(Benzyloxycarbonyl)-L-homophenylalaninal (6b).** Yield 94%. Pale yellow oil. ^1H NMR (DMSO-d_6) δ 1.75 (m, 1H), 2.02 (m, 1H), 2.55–2.73 (m, 2H), 3.91 (m, 1H), 5.08 (s, 2H), 7.17–7.45 (m, 10H), 7.85 (d, 1H, $J=7.2$ Hz), 9.49 (s, 1H). HRMS (MALDI) calcd for $C_{18}H_{19}NO_3$ $(M+Na)^+$, 320.1263, found, 320.1285.

***N*-(Benzyloxycarbonyl)-3-(2-naphthyl)-L-alaninal (6c).** Yield 57%. Colorless crystals. Mp 65–67 °C. ^1H NMR (DMSO-d_6) δ 2.82 (m, 1H), 3.02 (dd, 1H, $J=13.7$, 5.3 Hz), 3.74 (m, 1H), 4.88–4.99 (m, 2H), 7.14–7.52 (m, 8H), 7.72 (s, 1H), 7.80–7.89 (m, 4H), 9.63 (s, 1H). HRMS (MALDI) calcd for $C_{21}H_{19}NO_3$ $(M+Na)^+$, 356.1263, found, 356.1268.

***N*-(Benzyloxycarbonyl)-D-phenylalaninal (6d).** Yield 81%. Colorless crystals. Mp 66–67 °C. ^1H NMR (DMSO-d_6) δ 2.72 (dd, 1H, $J=14.0$, 10.4 Hz), 3.15 (dd, 1H, $J=14.0$, 4.7 Hz), 4.20 (m, 1H), 4.96–5.05 (m, 2H), 7.19–7.39 (m, 10H), 7.75 (d, 1H, $J=7.8$ Hz), 9.57 (s, 1H). Anal. calcd for $C_{17}H_{17}NO_3$: C, 72.07; H, 6.05; N, 4.94; found: C, 72.09; H, 6.01; N, 5.05.

Typical procedure for the preparation of compounds 7a–d

***N*-((1*S*)-1-(2,5-Dioxolanyl)-2-phenylethyl)(benzyloxy)formamide (7a).** To a solution of **6a** (29 g, 100 mmol) in toluene (500 mL) were added ethylene glycol (31 g, 510 mmol) and pyridinium *p*-toluenesulfonate (5.1 g, 20 mmol). The mixture was stirred at 80 °C for 18 h. The mixture was washed with 1 M HCl, saturated $NaHCO_3$ and saturated NaCl, dried over $MgSO_4$ and concentrated in vacuo. The residue was crystallized from hexane/EtOAc to give **7a** (33 g, 75%) as colorless crystals. Mp 88–92 °C. ^1H NMR (DMSO-d_6) δ 2.62 (dd, 1H, $J=14.0$, 11.0 Hz), 2.84 (dd, 1H, $J=14.0$, 3.8 Hz), 3.78–3.96 (m, 5H), 4.82 (d, 1H, $J=3.6$ Hz), 4.90 (d, 1H, $J=12.9$ Hz), 4.96 (d, 1H, $J=13.2$ Hz), 7.19–7.35 (m, 11H). Anal. calcd for $C_{19}H_{21}NO_4$: C, 69.71; H, 6.47; N, 4.28; found: C, 69.68; H, 6.49; N, 3.99.

***N*-((1*S*)-1-(2,5-Dioxolanyl)-3-phenylpropyl)(benzyloxy)formamide (7b).** Yield 24%. Colorless crystals. Mp 111–112 °C. ^1H NMR (DMSO-d_6) δ 1.59–1.78 (m, 2H), 2.49 (m, 1H), 2.65 (m, 1H), 3.57 (m, 1H), 3.75–3.87 (m, 4H), 4.74 (d, 1H, $J=3.9$ Hz), 5.05 (s, 2H), 7.15–7.38 (m, 11H). Anal. calcd for $C_{20}H_{23}NO_4$: C, 70.36; H, 6.79; N, 4.10; found: C, 70.55; H, 6.78; N, 3.96.

***N*-((1*S*)-1-(2,5-Dioxolanyl)-2-(2-naphthyl)ethyl)(benzyloxy)formamide (7c).** Yield 82%. Colorless crystals. Mp 128–129 °C. ^1H NMR (DMSO-d_6) δ 2.79 (dd, 1H, $J=13.8$, 10.8 Hz), 3.02 (dd, 1H, $J=14.0$, 3.8 Hz), 3.83-4.04 (m, 5H), 4.82–4.94 (m, 3H), 7.09–7.22 (m, 5H), 7.38–7.51 (m, 4H), 7.73 (s, 1H), 7.80–7.90 (m, 3H). HRMS (MALDI) calcd for $C_{23}H_{23}NO_4$ $(M+Na)^+$, 400.1525, found, 400.1531.

***N*-((1*R*)-1-(2,5-Dioxolanyl)-2-phenylethyl)(benzyloxy)formamide (7d).** Yield 71%. Colorless crystals. Mp 121–122 °C. ^1H NMR (DMSO-d_6) δ 2.62 (dd, 1H, $J=14.1$, 10.8 Hz), 2.84 (dd, 1H, $J=14.1$, 4.1 Hz), 3.78–3.96 (m, 5H), 4.82 (d, 1H, $J=3.6$ Hz), 4.90 (d, 1H, $J=12.9$ Hz), 4.96 (d, 1H, $J=12.9$ Hz), 7.19–7.34 (m, 11H). Anal. calcd for $C_{19}H_{21}NO_4$: C, 69.71; H, 6.47; N, 4.28; found: C, 69.68; H, 6.49; N, 4.32.

Typical procedure for the preparation of compounds 8a–d

(1*S*)-1-(2,5-Dioxolanyl)-2-phenylethylamine (8a). Compound **7a** (32 g, 96 mmol) was dissolved in EtOAc (250 mL) and hydrogenated at room temperature under atmosphere pressure over Pd/C powder (10%) (15 g). After stirring for 18 h, Pd/C was filtered off, and the filtrate was concentrated in vacuo to give **8a** (19 g, 95%) as a colorless oil. ^{1}H NMR (DMSO-d_6) δ 1.21 (s, 2H), 2.44 (dd, 1H, J = 13.3, 9.3 Hz), 2.79 (dd, 1H, J = 13.3, 4.4 Hz), 2.88 (m, 1H), 3.80–3.97 (m, 4H), 4.61 (d, 1H, J = 3.9 Hz), 7.15–7.31 (m, 5H). HRMS (MALDI) calcd for $C_{11}H_{15}NO_2$ $(M+Na)^+$, 216.1001, found, 216.1008.

(1*S*)-1-(2,5-Dioxolanyl)-3-phenylpropylamine (8b). Yield 82%. Colorless oil. ^{1}H NMR (DMSO-d_6) δ 1.32 (s, 2H), 1.45 (m, 1H), 1.73 (m, 1H), 2.54–2.56 (m, 2H), 2.79 (m, 1H), 3.75–3.90 (m, 4H), 4.58 (d, 1H, J = 4.2 Hz), 7.13–7.30 (m, 5H). HRMS (MALDI) calcd for $C_{12}H_{17}NO_2$ $(M+Na)^+$, 230.1157, found, 230.1166.

(1*S*)-1-(2,5-Dioxolanyl)-2-(2-naphthyl)ethylamine (8c). Yield 97%. Colorless oil. ^{1}H NMR (DMSO-d_6) δ 1.57 (s, 2H), 2.63 (dd, 1H, J = 13.1, 8.9 Hz), 2.94–3.05 (m, 2H), 3.79–4.02 (m, 4H), 4.68 (d, 1H, J = 3.6 Hz), 7.41–7.51 (m, 3H), 7.73 (s, 1H), 7.82–7.88 (m, 3H). HRMS (MALDI) calcd for $C_{15}H_{17}NO_2$ $(M+Na)^+$, 266.1157, found, 266.1163.

(1*R*)-1-(2,5-Dioxolanyl)-2-phenylethylamine (8d). Yield 75%. Colorless oil. ^{1}H NMR (DMSO-d_6) δ 1.25 (s, 2H), 2.44 (dd, 1H, J = 13.2, 9.3 Hz), 2.79 (dd, 1H, J = 13.4, 4.4 Hz), 2.88 (m, 1H), 3.79–3.97 (m, 4H), 4.61 (d, 1H, J = 3.9 Hz), 7.15–7.31 (m, 5H). HRMS (MALDI) calcd for $C_{11}H_{15}NO_2$ $(M+Na)^+$, 216.1001, found, 216.1011.

Typical procedure for the preparation of compounds 9a–d, 11a–b and 13a

(2*S*)-*N*-((1*S*)-1-(2,5-Dioxolanyl)-2-phenylethyl)-2-hydroxy-4-methylpentanamide (9a). Compound **8a** (15 g, 78 mmol), L-leucic acid (10 g, 78 mmol), HOBt (12 g, 85 mmol) and Et_3N (8.6 g, 85 mmol) were dissolved in DMF (120 mL). A suspension of EDC (16 g, 85 mmol) in CH_2Cl_2 (40 mL) was added to the mixture under the ice cool condition. The mixture was stirred at room temperature for 18 h and concentrated in vacuo. The mixture was diluted with EtOAc, and the solution was washed with 1 M HCl, saturated $NaHCO_3$ and saturated NaCl, dried over $MgSO_4$ and concentrated in vacuo. The residue was crystallized from EtOAc to give **9a** (18 g, 75%) as colorless crystals. Mp 104–107 °C. ^{1}H NMR (DMSO-d_6) δ 0.78 (d, 6H, J = 6.6 Hz), 1.05–1.20 (m, 2H), 1.59 (m, 1H), 2.69 (dd, 1H, J = 14.1, 10.2 Hz), 2.86 (dd, 1H, J = 14.1, 4.5 Hz), 3.74-3.97 (m, 5H), 4.16 (m, 1H), 4.83 (d, 1H, J = 3.3 Hz), 5.40 (d, 1H, J = 5.7 Hz), 7.13–7.27 (m, 5H), 7.34 (d, 1H, J = 9.6 Hz). Anal. calcd for $C_{17}H_{25}NO_4$: C, 66.43; H, 8.20; N, 4.56; found: C, 66.60; H, 8.30; N, 4.27.

(2*S*)-*N*-((1*S*)-1-(2,5-Dioxolanyl)-3-phenylpropyl)-2-hydroxy-4-methylpentanamide (9b). Yield 82%. Colorless crystals. Mp 87–88 °C. ^{1}H NMR (DMSO-d_6) δ 0.89 (d, 3H, J = 6.6 Hz), 0.90 (d, 3H, J = 6.6 Hz), 1.36-1.53 (m, 2H), 1.64-1.84 (m, 3H), 2.46 (m, 1H), 2.60 (m, 1H), 3.75-3.97 (m, 6H), 4.80 (d, 1H, J = 3.3 Hz), 5.47 (d, 1H, J = 6.3 Hz), 7.14–7.19 (m, 3H), 7.25-7.30 (m, 2H), 7.41 (d, 1H, J = 9.3 Hz). HRMS (MALDI) calcd for $C_{18}H_{27}NO_4$ $(M+Na)^+$, 344.1838, found, 344.1832.

(2*S*)-*N*-((1*S*)-1-(2,5-Dioxolanyl)-2-(2-naphthyl)ethyl)-2-hydroxy-4-methylpentanamide (9c). Yield 54%. Colorless crystals. Mp 142–143 °C. ^{1}H NMR (DMSO-d_6) δ 0.61–0.65 (m, 6H), 0.94–1.01 (m, 2H), 1.46 (m, 1H), 2.85 (dd, 1H, J = 13.7, 10.4 Hz), 3.05 (dd, 1H, J = 14.0, 4.4 Hz), 3.72 (m, 1H), 3.81–3.99 (m, 4H), 4.27 (m, 1H), 4.90 (d, 1H, J = 3.6 Hz), 5.41 (d, 1H, J = 5.7 Hz), 7.36–7.48 (m, 4H), 7.67 (s, 1H), 7.77–7.86 (m, 3H). Anal. calcd for $C_{21}H_{27}NO_4$: C, 70.56; H, 7.61; N, 3.92; found: C, 70.21; H, 7.64; N, 3.80.

(2*S*)-*N*-((1*R*)-1-(2,5-Dioxolanyl)-2-phenylethyl)-2-hydroxy-4-methylpentanamide (9d). Yield 81%. Colorless crystals. Mp 121–122 °C. ^{1}H NMR (DMSO-d_6) δ 0.80 (d, 3H, J = 3.9 Hz), 0.83 (d, 3H, J = 3.9 Hz), 1.15–1.34 (m, 2H), 1.62 (m, 1H), 2.70 (dd, 1H, J = 14.1, 10.1 Hz), 2.86 (dd, 1H, J = 14.1, 4.5 Hz), 3.72–3.98 (m, 5H), 4.14 (m, 1H), 4.83 (d, 1H, J = 3.3 Hz), 5.28 (d, 1H, J = 6.3 Hz), 7.14–7.27 (m, 5H), 7.44 (d, 1H, J = 9.3 Hz). Anal. calcd for $C_{17}H_{25}NO_4$: C, 66.43; H, 8.20; N, 4.56; found: C, 66.53; H, 8.24; N, 4.46.

(2*S*)-*N*-((1*S*)-1-(2,5-Dioxolanyl)-2-phenylethyl)-2-hydroxy-3-methylbutanamide (11a). Yield 92%. Colorless crystals. Mp 91–92 °C. ^{1}H NMR (DMSO-d_6) δ 0.46 (d, 3H, J = 6.9 Hz), 0.74 (d, 3H, J = 7.2 Hz), 1.79 (m, 1H), 2.70 (dd, 1H, J = 13.9, 10.5 Hz), 2.87 (dd, 1H, J = 13.9, 4.4 Hz), 3.60 (dd, 1H, J = 6.0, 3.6 Hz), 3.79–3.97 (m, 4H), 4.22 (m, 1H), 4.83 (d, 1H, J = 3.6 Hz), 5.31 (d, 1H, J = 5.7 Hz), 7.13–7.26 (m, 5H), 7.33 (d, 1H, J = 9.3 Hz). Anal. calcd for $C_{16}H_{23}NO_4$: C, 65.51; H, 7.90; N, 4.77; found: C, 65.36; H, 7.84; N, 4.54.

(2*S*)-*N*-((1*S*)-1-(2,5-Dioxolanyl)-3-phenylpropyl)-2-hydroxy-3-methylbutanamide (11b). Yield 84%. Colorless crystals. Mp 104–111 °C. ^{1}H NMR (DMSO-d_6) δ 0.82 (d, 3H, J = 6.9 Hz), 0.93 (d, 3H, J = 6.9 Hz), 1.62–1.86 (m, 2H), 2.02 (m, 1H), 2.47 (m, 1H), 2.62 (m, 1H), 3.73–4.00 (m, 6H), 4.81 (d, 1H, J = 3.6 Hz), 5.41 (d, 1H, J = 6.3 Hz), 7.14–7.19 (m, 3H), 7.25–7.27 (m, 2H), 7.42 (d, 1H, J = 9.3 Hz). Anal. calcd for $C_{17}H_{25}NO_4$: C, 66.43; H, 8.20; N, 4.56; found: C, 66.73; H, 8.18; N, 5.00.

(2*S*)-*N*-((1*S*)-1-(2,5-Dioxolanyl)-2-phenylethyl)-2-hydroxy-3-phenylpropanamide (13a). Yield 82%. Colorless crystals. Mp 119–121 °C. ^{1}H NMR (DMSO-d_6) δ 2.43 (dd, 1H, J = 13.7, 8.9 Hz), 2.65–2.85 (m, 3H), 3.77–4.01 (m, 5H), 4.17 (m, 1H), 4.81 (d, 1H, J = 3.6 Hz), 5.55 (d, 1H, J = 6.0 Hz), 7.12-7.29 (m, 10H), 7.45 (d, 1H, J = 9.6 Hz). Anal. calcd for $C_{20}H_{23}NO_4$: C, 70.36; H, 6.79; N, 4.10; found: C, 70.23; H, 6.71; N, 4.01.

Typical procedure for the preparation of compounds 10a–d, 12a–b and 14a

(2*S*,5*S*)-5-Benzyl-6-hydroxy-2-(2-methylpropyl)-3-mor-

pholinone (10a). To a solution of **9a** (2.0 g, 6.5 mmol) in THF (150 mL) was added 6 M HCl (150 mL). The mixture was stirred at room temperature for 18 h. After removal of THF in vacuo, the product was extracted with EtOAc, and the solution was washed with saturated $NaHCO_3$ and saturated NaCl, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified with HPLC system (column: YMC-Pack ODS-A 250×20 mm, mobile phase: CH_3CN/H_2O/TFA = 20:80:0.1). The main fractions were collected, and extracted with EtOAc. The organic layer was washed with saturated $NaHCO_3$ and saturated NaCl, dried over $MgSO_4$ and concentrated in vacuo. The residue was crystallized from hexane/acetone to give **10a** (0.50 g, 29%) as colorless crystals. Mp 84–85 °C. ^{1}H NMR (DMSO-d_6) δ 0.88 (d, 3H, J = 6.6 Hz), 0.90 (d, 3H, J = 7.2 Hz), 1.44 (m, 1H), 1.59 (m, 1H), 1.79 (m, 1H), 2.71–2.81 (m, 2H), 3.37 (m, 1H), 4.09 (dd, 1H, J = 9.8, 3.5 Hz), 4.84 (d, 1H, J = 4.1 Hz), 6.65 (d, 1H, J = 4.1 Hz), 7.17–7.34 (m, 5H), 7.79 (d, 1H, J = 4.5 Hz). HRMS (ESI) calcd for $C_{15}H_{21}NO_3$ $(M+Na)^+$, 286.1419, found, 286.1428. $[\alpha]_D^{25}$ −75.8° (*c* 0.219, DMSO).

(2*S*,5*S*)-6-Hydroxy-2-(2-methylpropyl)-5-(2-phenylethyl)-3-morpholinone (10b). Yield 24%. Colorless oil. ^{1}H NMR (DMSO-d_6) δ 0.87 (d, 3H, J = 6.3 Hz), 0.90 (d, 3H, J = 6.6 Hz), 1.46–1.83 (m, 5H), 2.54–2.74 (m, 2H), 3.11 (m, 1H), 4.10 (dd, 1H, J = 9.6, 3.6), 4.96 (d, 1H, J = 4.2 Hz), 6.67 (d, 1H, J = 4.2 Hz), 7.15–7.31 (m, 5H), 8.01 (d, 1H, J = 3.6 Hz). HRMS (ESI) calcd for $C_{16}H_{23}NO_3$ $(M+Na)^+$, 300.1576, found, 300.1599. $[\alpha]_D^{25}$ −80.4° (*c* 0.214, DMSO).

(2*S*,5*S*)-6-Hydroxy-2-(2-methylpropyl)-5-(2-naphthylmethyl)-3-morpholinone (10c). Yield 43%. Colorless oil. ^{1}H NMR (DMSO-d_6) δ 0.82–0.88 (m, 6H), 1.41 (m, 1H), 1.59 (m, 1H), 1.78 (m, 1H), 2.89–2.97 (m, 2H), 3.49 (m, 1H), 4.11 (dd, 1H, J = 9.8, 3.5 Hz), 4.94 (d, 1H, J = 3.9 Hz), 6.67 (d, 1H, J = 4.5 Hz), 7.35–7.53 (m, 3H), 7.71 (s, 1H), 7.76–7.90 (m, 4H). HRMS (ESI) calcd for $C_{19}H_{23}NO_3$ $(M+Na)^+$, 336.1576, found, 336.1541.

(2*S*,5*R*)-5-Benzyl-6-hydroxy-2-(2-methylpropyl)-3-morpholinone (10d). Yield 9.9%. Colorless oil. ^{1}H NMR (DMSO-d_6) δ 0.85 (d, 3H, J = 4.2 Hz), 0.87 (d, 3H, J = 4.5 Hz), 1.47–1.65 (m, 2H), 1.74 (m, 1H), 2.71–2.82 (m, 2H), 3.41 (m, 1H), 3.66/4.67 (m, 1H), 3.86/4.10 (dd, 1H, J = 9.1, 3.8 Hz), 6.79/6.97 (d, 1H, J = 4.5/6.0 Hz), 7.20–7.33 (m, 5H), 7.62 (d, 1H, J = 4.5 Hz). HRMS (ESI) calcd for $C_{15}H_{21}NO_3$ $(M+Na)^+$, 286.1419, found, 286.1485. $[\alpha]_D^{25}$ −95.0 ° (*c* 0.200, DMSO).

(2*S*,5*S*)-5-Benzyl-6-hydroxy-2-(1-methylethyl)-3-morpholinone (12a). Yield 49%. White solid. Mp 39–40 °C. ^{1}H NMR (DMSO-d_6) δ 0.92 (d, 3H, J = 6.6 Hz), 0.99 (d, 3H, J = 6.9 Hz), 2.30 (m, 1H), 2.75–2.78 (m, 2H), 3.67 (m, 1H), 3.98 (m, 1H), 4.88 (d, 1H, J = 3.9 Hz), 6.67 (m, 1H), 7.16–7.35 (m, 5H), 7.81 (m, 1H). HRMS (ESI) calcd for $C_{14}H_{19}NO_3$ $(M+Na)^+$, 272.1263, found, 272.1244. $[\alpha]_D^{25}$ −108° (*c* 0.209, DMSO).

(2*S*,5*S*)-6-Hydroxy-2-(1-methylethyl)-5-(2-phenylethyl)-3-morpholinone (12b). Yield 21%. Colorless oil. ^{1}H NMR (DMSO-d_6) δ 0.84 (d, 3H, J = 6.9 Hz), 0.96 (d, 3H, J = 7.2 Hz), 1.63–1.85 (m, 2H), 2.27 (m, 1H), 2.53–2.73 (m, 2H), 3.06 (m, 1H), 3.96 (m, 1H), 5.03 (d, 1H, J = 3.6 Hz), 6.68 (d, 1H, J = 4.2 Hz), 7.15–7.31 (m, 5H), 8.05 (d, 1H, J = 3.9 Hz). HRMS (ESI) calcd for $C_{15}H_{21}NO_3$ $(M+Na)^+$, 286.1419, found, 286.1439. $[\alpha]_D^{25}$ −66.1° (*c* 0.233, DMSO).

(2*S*,5*S*)-2,5-Dibenzyl-6-hydroxy-3-morpholinone (14a). Yield 63%. Colorless crystals. Mp 165–166 °C. ^{1}H NMR (DMSO-d_6) δ 2.22–2.38 (m, 2H), 2.92–3.05 (m, 2H), 3.24 (m, 1H), 4.34 (dd, 1H, J = 6.3, 4.2 Hz), 4.77 (d, 1H, J = 3.6 Hz), 6.66 (d, 1H, J = 3.9 Hz), 6.99 (d, 2H, J = 7.5), 7.17–7.33 (m, 8H), 7.83 (d, 1H, J = 4.5 Hz). Anal. calcd for $C_{18}H_{19}NO_3$: C, 72.71; H, 6.44; N, 4.71; found: C, 72.72; H, 6.67; N, 4.70.

***N*-(Benzyloxycarbonyl)-L-tyrosine methyl ester (16a).** To a solution of L-tyrosine methyl ester hydrochloride (**15**) (25 g, 108 mmol) in THF (250 mL) was added Cbz-OSu (27 g, 108 mmol) and Et_3N (22 g, 216 mmol) under the ice cool condition. The mixture was stirred at room temperature for 18 h. After concentration in vacuo, the residue was dissolved in EtOAc, and the solution was washed with 1 M HCl, saturated $NaHCO_3$ and saturated NaCl, dried over $MgSO_4$ and concentrated in vacuo. The residue was crystallized from hexane to give **16a** (32 g, 90%) as colorless crystals. Mp 86 °C. ^{1}H NMR (DMSO-d_6) δ 2.76 (dd, 1H, J = 13.7, 10.1 Hz), 2.92 (dd, 1H, J = 13.8, 5.1 Hz), 3.61 (s, 3H), 4.19 (m, 1H), 4.99 (s, 2H), 6.67 (d, 2H, J = 8.1 Hz), 7.03 (d, 2H, J = 8.4 Hz), 7.26–7.40 (m, 5H), 7.76 (d, 1H, J = 8.1 Hz), 9.24 (s, 1H). Anal. calcd for $C_{18}H_{19}O_5$: C, 65.64; H, 5.81; N, 4.25; found: C, 65.31; H, 5.79; N, 4.15.

***N*-(Benzyloxycarbonyl)-*O*-methyl-L-tyrosine methyl ester (16b).** To a solution of **16a** (1.5 g, 4.6 mmol) in dry acetone (20 mL), anhydrous K_2CO_3 (1.3 g, 9.1 mmol) and dimethyl sulfate (0.60 g, 4.6 mmol) were added, and the mixture was refluxed for 4 h. After cooling to room temperature, water (1.0 mL) was added thereto, and the mixture was stirred for 2 h to destroy any remaining dimethyl sulfate. The inorganic material was filtered off, and the acetone was removed in vacuo. The residue was dissolved in CH_2Cl_2, and the solution was washed with water, dried over $MgSO_4$ and concentrated in vacuo. The residue was crystallized from hexane to give **16b** (1.4 g, 90%) as colorless crystals. Mp 84–88 °C. ^{1}H NMR (DMSO-d_6) δ 2.80 (dd, 1H, J = 13.5, 10.5 Hz), 2.97 (dd, 1H, J = 13.4, 5.0 Hz), 3.62 (s, 3H), 3.72 (s, 3H), 4.22 (m, 1H), 4.99 (s, 2H), 6.84 (d, 2H, J = 8.4 Hz), 7.16 (d, 2H, J = 8.4 Hz), 7.26–7.38 (m, 5H), 7.78 (d, 1H, J = 8.1 Hz). HRMS (MALDI) calcd for $C_{19}H_{21}NO_5$ $(M+Na)^+$, 366.1318, found, 366.1322.

Typical procedure for the preparation of compounds 16c–d

***N*-(Benzyloxycarbonyl)-*O*-butyl-L-tyrosine methyl ester (16c).** To a solution of **16a** (18 g, 55 mmol) in DMF (70 mL) was added K_2CO_3 (15 g, 110 mmol) and *n*-butyl bromide (11 g, 82 mmol). The mixture was stirred

at room temperature for 18 h. The inorganics were filtered off, and the filtrate was concentrated in vacuo. The residue was dissolved in EtOAc, and the solution was washed with 1 M HCl and saturated NaCl, dried over $MgSO_4$ and concentrated in vacuo to give **16c** (20 g, 95%) as a colorless oil. ^{1}H NMR (DMSO-d_6) δ 0.93 (t, 3H, J = 7.2 Hz), 1.37–1.49 (m, 2H), 1.63–1.72 (m, 2H), 2.79 (dd, 1H, J = 13.5, 10.2 Hz), 2.97 (dd, 1H, J = 13.7, 5.0 Hz), 3.57–3.62 (m, 3H), 3.92 (t, 2H, J = 6.5 Hz), 4.21 (m, 1H), 4.99 (s, 2H), 6.81–6.84 (m, 2H), 7.12–7.15 (m, 2H), 7.26–7.38 (m, 5H), 7.78 (d, 1H, J = 8.1 Hz). HRMS (MALDI) calcd for $C_{22}H_{27}NO_5$ $(M+Na)^+$, 408.1787, found, 408.1759.

***N*-(Benzyloxycarbonyl)-*O*-cyclohexylmethyl-L-tyrosine methyl ester (16d).** Yield 77%. Colorless oil. ^{1}H NMR (DMSO-d_6) δ 0.96–1.81 (m, 11H), 2.77 (m, 1H), 2.93 (m, 1H), 3.56–3.63 (m, 3H), 3.73 (d, 2H, J = 5.7 Hz), 4.19 (m, 1H), 4.97–4.98 (m, 2H), 6.65–6.83 (m, 2H), 7.01–7.14 (m, 2H), 7.28–7.32 (m, 5H), 7.74–7.79 (m, 1H). HRMS (MALDI) calcd for $C_{25}H_{31}NO_5$ $(M+Na)^+$, 448.2100, found, 448.2071.

Typical procedure for the preparation of compounds 17a–d

***N*-(Benzyloxycarbonyl)-L-tyrosinol (17a).** To a solution of **16a** (18 g, 55 mmol) in THF (100 mL) was added anhydrous LiCl (7.0 g, 160 mmol) and $NaBH_4$ (6.2 g, 160 mmol). After addition of EtOH (200 mL), the mixture was stirred at room temperature for 18 h. The mixture was adjusted to pH 4 by addition of 10% aqueous citric acid, and concentrated in vacuo. The residue was extracted with CH_2Cl_2, and the solution was dried over $MgSO_4$ and concentrated in vacuo. The crude product was crystallized from hexane to give **17a** (16 g, 91%) as colorless crystals. Mp 67–68 °C. ^{1}H NMR (DMSO-d_6) δ 2.46 (m, 1H), 2.72 (dd, 1H, J = 14.0, 5.0 Hz), 3.34 (m, 1H), 3.51–3.61 (m, 2H), 4.69 (t, 1H, J = 5.3 Hz), 4.96 (dd, 2H, J = 17.4, 12.6 Hz), 6.63–6.67 (m, 2H), 6.97–7.04 (m, 3H), 7.25–7.37 (m, 5H), 9.13 (s, 1H). Anal. calcd for $C_{17}H_{19}NO_4$: C, 67.76; H, 6.36; N, 4.65; found: C, 67.99; H, 6.32; N, 4.54.

***N*-(Benzyloxycarbonyl)-*O*-methyl-L-tyrosinol (17b).** Yield 75%. Colorless crystals. Mp 82–84 °C. ^{1}H NMR (DMSO-d_6) δ 2.51 (m, 1H), 2.78 (dd, 1H, J = 14.0, 5.3 Hz), 3.25–3.40 (m, 2H), 3.60 (m, 1H), 3.72 (s, 3H), 4.72 (t, 1H, J = 5.6 Hz), 4.96 (dd, 2H, J = 15.6, 12.9 Hz), 6.80–6.85 (m, 2H), 7.05-7.13 (m, 3H), 7.25–7.36 (m, 5H). Anal. calcd for $C_{18}H_{21}NO_4$: C, 68.55; H, 6.71; N, 4.44; found: C, 68.41; H, 6.63; N, 4.37.

***N*-(Benzyloxycarbonyl)-*O*-butyl-L-tyrosinol (17c).** Yield 73%. Colorless crystals. Mp 65 °C. ^{1}H NMR (DMSO-d_6) δ 0.93 (t, 3H, J = 7.4 Hz), 1.37–1.49 (m, 2H), 1.63–1.73 (m, 2H), 2.50 (m, 1H), 2.77 (dd, 1H, J = 13.5, 5.1 Hz), 3.26–3.41 (m, 2H), 3.61 (m, 1H), 3.92 (t, 2H, J = 6.3 Hz), 4.72 (t, 1H, J = 5.6 Hz), 4.96 (dd, 2H, J = 16.8, 12.6 Hz), 6.78–6.83 (m, 2H), 7.05–7.15 (m, 3H), 7.24–7.37 (m, 5H). Anal. calcd for $C_{21}H_{27}NO_4$: C, 70.56; H, 7.61; N, 3.92; found: C, 70.27; H, 7.40; N, 3.75.

***N*-(Benzyloxycarbonyl)-*O*-cyclohexylmethyl-L-tyrosinol (17d).** Yield 42%. Colorless crystals. Mp 56–57 °C. ^{1}H NMR (DMSO-d_6) δ 0.97–1.82 (m, 11H), 2.49 (m, 1H), 2.77 (dd, 1H, J = 14.0, 5.0 Hz), 3.25–3.40 (m, 2H), 3.60 (m, 1H), 3.72 (d, 2H, J = 6.0 Hz), 4.73 (t, 1H, J = 5.6 Hz), 4.90–5.00 (m, 2H), 6.79–6.81 (m, 2H), 7.01–7.10 (m, 3H), 7.24–7.36 (m, 5H). HRMS (MALDI) calcd for $C_{24}H_{31}NO_4$ $(M+Na)^+$, 420.2151, found, 420.2093.

Typical procedure for the preparation of compounds 18a–d

***N*-(Benzyloxycarbonyl)-L-tyrosinal (18a).** Compound **17a** (15 g, 50 mmol) was dissolved in DMSO (60 mL) and CH_2Cl_2 (30 mL), and cooled under the ice cool condition. *N,N*-Diisopropylethylamine (19 g, 150 mmol) and a suspension of SO_3/pyridine (24 g, 150 mmol) in DMSO (30 mL) were added thereto. The mixture was stirred for 30 min under the same conditions. The reaction mixture was diluted with EtOAc, and the solution was washed with 1 M HCl, saturated $NaHCO_3$ and saturated NaCl, dried over $MgSO_4$ and concentrated in vacuo to give **18a** (15 g, 84%) as a colorless oil. ^{1}H NMR (DMSO-d_6) δ 2.59 (m, 1H), 3.03 (dd, 1H, J = 14.0, 4.1 Hz), 4.14 (m, 1H), 4.94–5.12 (m, 2H), 6.67–6.75 (m, 2H), 7.02–7.04 (m, 2H), 7.31–7.40 (m, 5H), 7.70 (d, 1H, J = 7.5 Hz), 9.24 (s, 1H), 9.56 (m, 1H). HRMS (MALDI) calcd for $C_{17}H_{17}NO_4$ $(M+Na)^+$, 322.1056, found, 322.1064.

***N*-(Benzyloxycarbonyl)-*O*-methyl-L-tyrosinal (18b).** Yield 81%. Colorless oil. ^{1}H NMR (DMSO-d_6) δ 2.66 (dd, 1H, J = 14.1, 10.2 Hz), 3.07 (dd, 1H, J = 14.1, 4.5 Hz), 3.72 (s, 3H), 4.15 (m, 1H), 4.92–5.05 (m, 2H), 6.81–6.86 (m, 2H), 7.11–7.19 (m, 2H), 7.23–7.39 (m, 6H), 9.56 (s, 1H). HRMS (MALDI) calcd for $C_{18}H_{19}NO_4$ $(M+Na)^+$, 336.1212, found, 336.1250.

***N*-(Benzyloxycarbonyl)-*O*-butyl-L-tyrosinal (18c).** Yield 74%. Colorless oil. ^{1}H NMR (DMSO-d_6) δ 0.93 (t, 3H, J = 7.2 Hz), 1.37–1.49 (m, 2H), 1.63–1.72 (m, 2H), 2.65 (dd, 1H, J = 14.0, 10.1 Hz), 3.07 (dd, 1H, J = 14.3, 4.7 Hz), 3.92 (t, 2H, J = 6.3 Hz), 4.15 (m, 1H), 5.01 (s, 2H), 6.80–6.85 (m, 2H), 7.11–7.14 (m, 2H), 7.27–7.39 (m, 5H), 7.71 (d, 1H, J = 7.5 Hz), 9.56 (s, 1H). HRMS (MALDI) calcd for $C_{21}H_{25}NO_4$ $(M+Na)^+$, 378.1682, found, 378.1627.

***N*-(Benzyloxycarbonyl)-*O*-cyclohexylmethyl-L-tyrosinal (18d).** Yield 83%. Colorless oil. ^{1}H NMR (DMSO-d_6) δ 0.97–1.31 (m, 5H), 1.64–1.82 (m, 6H), 2.65 (dd, 1H, J = 14.0, 10.1 Hz), 3.06 (dd, 1H, J = 14.1, 4.5 Hz), 3.73 (d, 2H, J = 6.3 Hz), 4.15 (m, 1H), 4.96–5.01 (m, 2H), 6.79–6.83 (m, 2H), 7.10–7.14 (m, 2H), 7.28–7.38 (m, 5H), 7.69–7.77 (m, 1H), 9.56 (s, 1H). HRMS (MALDI) calcd for $C_{24}H_{29}NO_4$ $(M+Na)^+$, 418.1995, found, 418.1991.

Typical procedure for the preparation of compounds 19a–d

***N*-((1*S*)-1-(2,5-Dioxolanyl)-2-(4-hydroxyphenyl)ethyl) (benzyloxy)formamide (19a).** To a solution of **18a** (13 g,

42 mmol) in toluene (150 mL) was added ethylene glycol (13 g, 209 mmol) and pyridinium *p*-toluenesulfonate (2.1 g, 8.4 mmol). The mixture was stirred at 80 °C for 18 h. The mixture was washed with 1 M HCl, saturated $NaHCO_3$ and saturated NaCl, dried over $MgSO_4$ and concentrated in vacuo to give **19a** (9.4 g, 66%) as a colorless oil. ^{1}H NMR (DMSO-d_6) δ 2.52 (m, 1H), 2.75 (m, 1H), 3.58 (m, 1H), 3.81 (m, 1H), 4.79 (d, 4H, J = 3.3 Hz), 4.89–5.01 (m, 2H), 6.64–6.67 (m, 2H), 7.00–7.02 (m, 2H), 7.19–7.35 (m, 6H), 9.16 (s, 1H). HRMS (MALDI) calcd for $C_{19}H_{21}NO_5$ $(M+Na)^+$, 366.1318, found, 366.1320.

***N*-((1*S*)-1-(2,5-Dioxolanyl)-2-(4-methoxyphenyl)ethyl)(benzyloxy)formamide (19b).** Yield 88%. Colorless crystals. Mp 56–59 °C. ^{1}H NMR (DMSO-d_6) δ 2.55 (m, 1H), 2.77 (dd, 1H, J = 13.7, 3.8 Hz), 3.72 (s, 3H), 3.76–3.95 (m, 5H), 4.80 (d, 1H, J = 3.9 Hz), 4.94 (dd, 2H, J = 19.4, 12.8 Hz), 6.79–6.83 (m, 2H), 7.12–7.14 (m, 2H), 7.19–7.35 (m, 6H). Anal. calcd for $C_{20}H_{23}NO_5$: C, 67.21; H, 6.49; N, 3.92; found: C, 66.85; H, 6.45; N, 3.86.

***N*-((1*S*)-2-(4-Butoxyphenyl)-1-(2,5-dioxolanyl)ethyl)(benzyloxy)formamide (19c).** Yield 76%. Colorless crystals. Mp 65–66 °C. ^{1}H NMR (DMSO-d_6) δ 0.93 (t, 3H, J = 7.4 Hz), 1.37–1.50 (m, 2H), 1.63–1.73 (m, 2H), 2.54 (m, 1H), 2.77 (dd, 1H, J = 14.1, 3.6 Hz), 3.72–3.95 (m, 7H), 4.79 (m, 1H), 4.94 (dd, 2H, J = 21.8, 13.1 Hz), 6.80 (d, 2H, J = 8.4 Hz), 7.11 (d, 2H, J = 8.4 Hz), 7.18–7.35 (m, 6H). HRMS (MALDI) calcd for $C_{23}H_{29}NO_5$ $(M+Na)^+$, 422.1944, found, 422.1939.

***N*-((1*S*)-2-(4-(Cyclohexylmethoxy)phenyl)-1-(2,5-dioxolanyl)ethyl)(benzyloxy)formamide (19d).** Yield 48%. Colorless crystals. Mp 61–62 °C. ^{1}H NMR (DMSO-d_6) δ 0.98–1.32 (m, 5H), 1.64–1.82 (m, 6H), 2.55 (m, 1H), 2.76 (dd, 1H, J = 13.8, 3.9 Hz), 3.71–3.77 (m, 3H), 3.79–3.95 (m, 4H), 4.80 (d, 1H, J = 3.9 Hz), 4.88–4.99 (m, 2H), 6.78–6.81 (m, 2H), 7.09–7.12 (m, 2H), 7.18–7.35 (m, 6H). HRMS (MALDI) calcd for $C_{26}H_{33}NO_5$ $(M+Na)^+$, 462.2257, found, 462.2210.

Typical procedure for the preparation of compounds 20a–d

(1*S*)-1-(2,5-Dioxolanyl)-2-(4-hydroxyphenyl)ethylamine (20a). Compound **19a** (9.4 g, 27 mmol) was dissolved in EtOAc (200 mL) and hydrogenated at room temperature under atmosphere pressure over Pd/C powder (10%) (6.0 g). After stirring for 18 h, Pd/C was filtered off, and the filtrate was concentrated in vacuo to give **20a** (5.7 g, 52%) as a colorless oil. ^{1}H NMR (DMSO-d_6) δ 2.34 (dd, 1H, J = 13.5, 9.0 Hz), 2.67 (m, 1H), 2.81 (m, 1H), 3.78–3.95 (m, 4H), 4.58 (d, 1H, J = 3.9 Hz), 6.65–6.69 (m, 2H), 6.98–7.03 (m, 2H). HRMS (MALDI) calcd for $C_{11}H_{15}NO_3$ $(M+Na)^+$, 232.0950, found, 232.0959.

(1*S*)-1-(2,5-Dioxolanyl)-2-(4-methoxyphenyl)ethylamine (20b). Yield 69%. Colorless oil. ^{1}H NMR (DMSO-d_6) δ 2.39 (dd, 1H, J = 13.5, 9.0 Hz), 2.72 (dd, 1H, J = 13.5, 4.5 Hz), 2.83 (m, 1H), 3.72 (s, 3H), 3.76–3.98 (m, 4H), 4.59 (d, 1H, J = 3.9 Hz), 6.81–6.86 (m, 2H), 7.11–7.16 (m, 2H). HRMS (MALDI) calcd for $C_{12}H_{17}NO_3$ $(M+Na)^+$, 246.1106, found, 246.1100.

(1*S*)-2-(4-Butoxyphenyl)-1-(2,5-dioxolanyl)ethylamine (20c). Yield 62%. Colorless oil. ^{1}H NMR (DMSO-d_6) δ 0.96 (t, 3H, J = 7.4 Hz), 1.40–1.52 (m, 2H), 1.66–1.75 (m, 2H), 2.43 (dd, 1H, J = 13.7, 9.2 Hz), 2.75 (dd, 1H, J = 13.5, 4.5 Hz), 2.87 (m, 1H), 3.79–3.86 (m, 2H), 3.89–4.02 (m, 4H), 4.63 (m, 1H), 6.84–6.87 (m, 2H), 7.13–7.16 (m, 2H). HRMS (MALDI) calcd for $C_{15}H_{23}NO_3$ $(M+Na)^+$, 288.1576, found, 288.1577.

(1*S*)-2-(4-(Cyclohexylmethoxy)phenyl)-1-(2,5-dioxolanyl)ethylamine (20d). Yield 81%. Colorless oil. ^{1}H NMR (DMSO-d_6) δ 0.96–1.31 (m, 6H), 1.63–1.81 (m, 5H), 2.37 (dd, 1H, J = 13.2, 9.0 Hz), 2.70 (dd, 1H, J = 13.5, 4.5 Hz), 2.81 (m, 1H), 3.71–3.73 (m, 2H), 3.75–3.98 (m, 4H), 4.58–4.59 (m, 1H), 6.80–6.83 (m, 2H), 7.09–7.12 (m, 2H). HRMS (MALDI) calcd for $C_{18}H_{27}NO_3$ $(M+Na)^+$, 328.1889, found, 328.1896.

Typical procedure for the preparation of compounds 21a–d

(2*S*)-*N*-((1*S*)-1-(2,5-Dioxolanyl)-2-(4-hydroxyphenyl)-ethyl)-2-hydroxy-4-methylpentanamide (21a). Compound **20a** (3.0 g, 14 mmol), L-leucic acid (1.9 g, 14 mmol), HOBt (2.1 g, 16 mmol) and Et_3N (1.6 g, 16 mmol) were dissolved in DMF (20 mL). A suspension of EDC (3.0 g, 14 mmol) in CH_2Cl_2 (10 mL) was added under the ice cool condition. The mixture was stirred at room temperature for 18 h and concentrated in vacuo. The mixture was diluted with EtOAc, and the solution was washed with 1 M HCl, saturated $NaHCO_3$ and saturated NaCl, dried over $MgSO_4$ and concentrated in vacuo. The residue was crystallized from EtOAc to give **21a** (2.3 g, 50%) as colorless crystals. Mp 71–74 °C. ^{1}H NMR (DMSO-d_6) δ 0.77–0.85 (m, 6H), 1.14–1.37 (m, 2H), 1.64 (m, 1H), 2.57 (m, 1H), 2.73 (m, 1H), 3.73–3.96 (m, 5H), 4.06 (m, 1H), 4.79 (d, 1H, J = 3.0 Hz), 5.35 (m, 1H), 6.61–6.64 (m, 2H), 6.95–6.99 (m, 2H), 7.31 (m, 1H), 9.12 (d, 1H, J = 6.0 Hz). HRMS (MALDI) calcd for $C_{17}H_{25}NO_5$ $(M+Na)^+$, 346.1631, found, 346.1635.

(2*S*)-*N*-((1*S*)-1-(2,5-Dioxolanyl)-2-(4-methoxyphenyl)-ethyl)-2-hydroxy-4-methylpentanamide (21b). Yield 33%. Colorless crystals. Mp 85–88 °C. ^{1}H NMR (DMSO-d_6) δ 0.79 (d, 6H, J = 6.3 Hz), 1.06–1.21 (m, 2H), 1.57 (m, 1H), 2.61 (dd, 1H, J = 14.0, 10.4 Hz), 2.79 (dd, 1H, J = 14.0, 4.7 Hz), 3.69 (s, 3H), 3.73–3.96 (m, 5H), 4.11 (m, 1H), 4.81 (d, 1H, J = 3.3 Hz), 5.39 (d, 1H, J = 5.7 Hz), 6.78–6.81 (m, 2H), 7.08–7.11 (m, 2H), 7.28 (d, 1H, J = 9.6 Hz). Anal. calcd for $C_{18}H_{27}NO_5$: C, 64.07; H, 8.07; N, 4.15; found: C, 63.66; H, 7.83; N, 4.25.

(2*S*)-*N*-((1*S*)-2-(4-Butoxyphenyl)-1-(2,5-dioxolanyl)-ethyl)-2-hydroxy-4-methylpentanamide (21c). Yield 72%. Colorless crystals. Mp 86 °C. ^{1}H NMR (DMSO-d_6) δ 0.78 (d, 6H, J = 6.9 Hz), 0.91 (t, 3H, J = 7.4 Hz), 1.09–1.15 (m, 2H), 1.35–1.47 (m, 2H), 1.51–1.71 (m, 3H), 2.60 (dd, 1H, J = 14.0, 10.4 Hz), 2.79 (dd, 1H, J = 13.8, 4.5 Hz), 3.73–3.95 (m, 7H), 4.11 (m, 1H), 4.81

(d, 1H, $J=3.3$ Hz), 5.39 (d, 1H, $J=5.7$ Hz), 6.77–6.79 (m, 2H), 7.06–7.09 (m, 2H), 7.27 (d, 1H, $J=9.3$ Hz). Anal. calcd for $C_{21}H_{33}NO_5$: C, 66.46; H, 8.76; N, 3.69; found: C, 66.11; H, 8.64; N, 3.42.

(2*S*)-*N*-((1*S*)-2-(4-(Cyclohexylmethoxy)phenyl)-1-(2,5-dioxolanyl)ethyl)-2-hydroxy-4-methylpentanamide (21d). Yield 73%. Colorless crystals. Mp 111–112 °C. ^{1}H NMR (DMSO-d_6) δ 0.77–0.85 (m, 6H), 0.92–1.34 (m, 7H), 1.53–1.80 (m, 7H), 2.56–2.65 (m, 1H), 2.74–2.82 (m, 1H), 3.69–3.97 (m, 7H), 4.09 (m, 1H), 4.81 (m, 1H), 5.26 (d, 1H, $J=6.3$ Hz), 6.76–6.79 (m, 2H), 7.05–7.09 (m, 2H), 7.26 (d, 1H, $J=9.3$ Hz). Anal. calcd for $C_{24}H_{37}NO_5$: C, 68.71; H, 8.89; N, 3.34; found: C, 68.42; H, 8.73; N, 3.56.

Typical procedure for the preparation of compounds 22a–d

(2*S*,5*S*)-6-Hydroxy-5-((4-hydroxyphenyl)methyl)-2-(2-methylpropyl)-3-morpholinone (22a). To a solution of **21a** (2.3 g, 7.1 mmol) in THF (200 mL) was added 6 M HCl (200 mL). The mixture was stirred at room temperature for 18 h. After removal of THF in vacuo, the product was extracted with EtOAc, and the solution was washed with saturated $NaHCO_3$ and saturated NaCl, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified with HPLC system (column: YMC-Pack ODS-A 250×20 mm, mobile phase: $CH_3CN/H_2O=25:75$). The main fractions were collected, and extracted with EtOAc. The organic layer was washed with saturated $NaHCO_3$ and saturated NaCl, dried over $MgSO_4$ and concentrated in vacuo to give **22a** (0.50 g, 25%) as a colorless oil. ^{1}H NMR (DMSO-d_6) δ 0.82–0.91 (m, 6H), 1.41–1.64 (m, 2H), 1.79 (m, 1H), 2.58–2.73 (m, 2H), 3.26 (m, 1H), 4.06 (m, 1H), 4.82 (d, 1H, $J=3.9$ Hz), 6.61 (d, 1H, $J=3.0$ Hz), 6.68–6.70 (m, 2H), 6.95–6.97 (m, 2H), 7.74 (d, 1H, $J=3.9$ Hz), 9.23 (m, 1H). HRMS (ESI) calcd for $C_{15}H_{21}NO_4$ $(M+Na)^+$, 302.1369, found, 302.1388.

(2*S*,5*S*)-6-Hydroxy-5-((4-methoxyphenyl)methyl)-2-(2-methylpropyl)-3-morpholinone (22b). Yield 35%. Colorless oil. ^{1}H NMR (DMSO-d_6) δ 0.82–0.91 (m, 6H), 1.44 (m, 1H), 1.59 (m, 1H), 1.55 (m, 1H), 2.60–2.82 (m, 2H), 3.30 (m, 1H), 3.70–3.73 (m, 3H), 4.09 (m, 1H), 4.84 (d, 1H, $J=4.5$ Hz), 6.64 (d, 1H, $J=4.2$ Hz), 6.81–6.90 (m, 2H), 7.07–7.12 (m, 2H), 7.77 (d, 1H, $J=4.2$ Hz). HRMS (ESI) calcd for $C_{16}H_{23}NO_4$ $(M+Na)^+$, 316.1525, found, 316.1573.

(2*S*,5*S*)-5-((4-Butoxyphenyl)methyl)-6-hydroxy-2-(2-methylpropyl)-3-morpholinone (22c). Yield 19%. Colorless oil. ^{1}H NMR (DMSO-d_6) δ 0.80–0.95 (m, 9H), 1.36–1.49 (m, 3H), 1.54–1.72 (m, 3H), 1.78 (m, 1H), 2.63–2.76 (m, 2H), 3.30 (m, 1H), 3.93 (t, 2H, $J=6.5$ Hz), 4.08 (dd, 1H, $J=9.6$, 3.6 Hz), 4.84 (s, 1H), 6.82–6.88 (m, 2H), 7.05–7.10 (m, 2H), 7.77 (d, 1H, $J=3.9$ Hz). HRMS (ESI) calcd for $C_{19}H_{29}NO_4$ $(M+Na)^+$, 358.1995, found, 358.1993.

(2*S*,5*S*)-5-((4-(Cyclohexylmethoxy)phenyl)methyl)-6-hydroxy-2-(2-methylpropyl)-3-morpholinone (22d). Yield 34%. Colorless oil. ^{1}H NMR (DMSO-d_6) δ 0.86–0.90 (m, 6H), 0.97–1.31 (m, 5H), 1.36–1.63 (m, 2H), 1.67–1.81 (m, 7H), 2.63–2.73 (m, 2H), 3.29 (m, 1H), 3.71–3.74 (m, 2H), 4.08 (m, 1H), 4.84 (d, 1H, $J=4.5$ Hz), 6.62 (d, 1H, $J=4.2$ Hz), 6.82–6.87 (m, 2H), 7.05–7.09 (m, 2H), 7.76 (d, 1H, $J=3.9$ Hz). HRMS (ESI) calcd for $C_{22}H_{33}NO_4$ $(M+Na)^+$, 398.2308, found, 398.2279.

***N*-(4-Methylpentanoyl)-L-phenylalaninol (23).** L-Phenylalaninol (**4a**) (1.0 g, 6.6 mmol), 4-methylpentanoic acid (0.84 g, 7.3 mmol), HOBt (0.98 g, 7.3 mmol) and Et_3N (0.74 g, 7.3 mmol) were dissolved in DMF (20 mL). A suspension of EDC (1.4 g, 7.3 mmol) in CH_2Cl_2 (20 mL) was added under the ice cool condition. The mixture was stirred at room temperature for 18 h and concentrated in vacuo. The mixture was diluted with EtOAc, and the solution was washed with 1 M HCl, saturated $NaHCO_3$ and saturated NaCl, dried over $MgSO_4$, and concentrated in vacuo. The residue was crystallized from EtOAc to give **23** (1.6 g, 91%) as colorless crystals. Mp 71–73 °C. ^{1}H NMR (DMSO-d_6) δ 0.79 (d, 3H, $J=6.3$ Hz), 0.80 (d, 3H, $J=6.3$ Hz), 1.22–1.40 (m, 3H), 2.00 (t, 2H, $J=7.5$ Hz), 2.58 (dd, 1H, $J=13.5$, 9.0 Hz), 2.85 (dd, 1H, $J=13.5$, 5.1 Hz), 3.23–3.39 (m, 2H), 3.90 (m, 1H), 4.74 (t, 1H, $J=5.0$ Hz), 7.13–7.28 (m, 5H), 7.60 (d, 1H, $J=8.4$ Hz). Anal. calcd for $C_{15}H_{23}NO_2$: C, 72.25; H, 9.30; N, 5.62; found: C, 71.95; H, 9.04; N, 5.56.

***N*-(4-Methylpentanoyl)-L-phenylalaninal (24).** Compound **23** (1.5 g, 6.0 mmol) was dissolved in DMSO (20 mL) and CH_2Cl_2 (20 mL), and cooled under the ice cool condition. *N,N*-Diisopropylethylamine (2.3 g, 18 mmol) and a suspension of SO_3/pyridine (2.9 g, 18 mmol) in DMSO (10 mL) were added thereto. The mixture was stirred for 30 min under the same conditions. The reaction mixture was diluted with EtOAc, and the solution was washed with 1 M HCl, saturated $NaHCO_3$ and saturated NaCl, dried over $MgSO_4$, and concentrated in vacuo. The residue was crystallized from hexane to give **24** (0.82 g, 55%) as colorless crystals. Mp 60–63 °C. ^{1}H NMR (DMSO-d_6) δ 0.81 (d, 3H, $J=6.6$ Hz), 0.82 (d, 3H, $J=6.6$ Hz), 1.30–1.46 (m, 3H), 2.10 (t, 2H, $J=7.7$ Hz), 2.75 (dd, 1H, $J=14.1$, 9.9 Hz), 3.14 (dd, 1H, $J=14.1$, 4.4 Hz), 4.27 (m, 1H), 7.18–7.31 (m, 5H), 8.28 (d, 1H, $J=7.2$ Hz), 9.48 (s, 1H). Anal. calcd for $C_{15}H_{21}NO_2$: C, 72.84; H, 8.56; N, 5.66; found: C, 73.03; H, 8.40; N, 5.54.

Inhibition assay for calpains

This inhibition assay was performed as described in the previous literature[11] using commercial μ-calpain (human erythrocyte, Calbiochem) and *m*-calpain (porcine kidney, Calbiochem). An assay solution including 0.5 mg/mL casein, 20 mM dithiothreitol, 50 mM Tris–HCl (pH 7.4) and 1.0 nmol of enzyme was used. The assay solution (200 μL) and DMSO (2.5 μL) including inhibitor of different concentration were placed in each well. Reaction was started by addition of 20 mM $CaCl_2$ (50 μL) in test well and 1 mM EDTA (50 μL) in blank well. After incubation for 60 min at 30 °C, the mixture (100 μL) was transferred to another plate in which H_2O (100 μL) and Bio-Rad protein assay dye reagent (50 μL)

were placed in each well. After incubation at room temperature for 15 min, the mixture was read OD at 595 nm with plate reader (Multiskan Multisoft, Labsystems). The percent inhibition was calculated from the difference of OD between the presence and absence of the compound. The IC_{50} was obtained by the graphical analysis of the concentration and the inhibition.

Inhibition assay for cathepsin B

10 μM *Z*-Phe-Arg-MCA (100 μL), H_2O (40 μL) and DMSO (2.5 μL) including inhibitor of different concentration were placed in each well. The reaction was started by addition of 0.015 unit cathepsin B (human liver, Sigma) solution (60 μL) at 30 °C, and the mixture was read fluorescence with plate reader (excitation; 360 nm, emission; 450 nm, Cytofluor series 4000, PerSeptive Biosystems) for 60 min. The percent inhibition was calculated from the difference of fluorescence between the inhibitor slope and control slope (no inhibitor). The IC_{50} was obtained by the graphical analysis of the concentration and the inhibition.

Casein zymogram

Casein zymogram experiment was performed as described in the previous literature.[9] *m*-Calpain (110 U/mL, rat recombinant, Calbiochem) was incubated in the presence or absence of 2.5 mM $CaCl_2$ in buffer (50 mM Tris–HCl (pH 7.4), 1 mM EGTA, and 3 mM dithiothreitol) at 30 °C for 10 min. On the other hand, *m*-calpain (110 U/mL) with inhibitor **10a** or E64 was incubated in the presence of $CaCl_2$ in buffer under the same conditions. After addition of excess EDTA, each incubation mixture was electrophoresed on casein gel. The gel was incubated with slow shaking for 2.5 h at room temperature in buffer (20 mM Tris–HCl (pH 7.4), 10 mM dithiothreitol and 20 mM $CaCl_2$), and stained with Coomassie Brilliant Blue to visualize caseinolysis.

In vitro corneal drug permeability

This experiment was performed using glass apparatus (modified Ussing chamber) as described in the previous literature.[10] Briefly, albino rabbits were sacrificed with pentobarbital overdosage and the whole eyes were enucleated. The corneas were excised and mounted in the chamber. Drug solution composed of 0.05% inhibitor (**10a** or **24**), 0.1% polysorbate 80, 0.1% $NaH_2PO_42H_2O$, 0.9% NaCl and NaOH (adjust to pH 7.0) was added to epithelial side (donor side) in the chamber. Receiver buffer composed of 0.0132% $CaCl_2$, 0.04% KCl, 0.02% $MgSO_4{\cdot}2H_2O$, 0.0187% $NaH_2PO_4{\cdot}2H_2O$, 0.787% NaCl, 0.1% glucose and NaOH (adjust to pH 7.2) was added to endothelial side (receiver side). Sample (100 μL) was withdrawn from receiver side at interval of 20 min, and analyzed for concentration of inhibitor by HPLC system.

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