New Type of Metalloproteinase Inhibitor: Design and Synthesis of New Phosphonamide-Based Hydroxamic Acids

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A series of phosphonamide-based hydroxamate derivatives were synthesized, and the inhibitory activities were evaluated against various metalloproteinases in order to clarify its selectivity profile. Among the four diastereomeric isomers resulting from the chirality at the C-3 and P atoms, the compound with a (*R*,*R*)-configuration both at the C-3 position and the phosphorus atom was found to be potently active, while the other diastereomeric isomers were almost inactive. A number of (*R*,*R*)-compounds synthesized here exhibited broad spectrum activities with nanomolar K_i values against MMP-1, -3, -9, and TACE and also showed nanomolar IC_{50} values against HB-EGF shedding in a cell-based inhibition assay. The modeling study using X-ray structure of MMP-3 suggested the possible binding mode of the phosphonamide-based inhibitors.

Introduction

A large number of zinc-dependent metalloproteinases (MPs) with various functions have been isolated and characterized recently. Matrix metalloproteinases (MMPs) are a family of such zinc-dependent metalloproteinases that play a significant physiological role in extracellular matrix remodeling.[1] The implication of MMPs in a number of pathological processes has been reported, and thus, they are considered to be important therapeutic targets for the treatment of a wide array of disease processes such as rheumatoid arthritis, tumor metastasis, multiple sclerosis, and congestive heart failure.[2]

The ADAMs (a disintegrin-like and metalloproteinase-containing protein) are also zinc-dependent metalloproteinases that have been implicated in procytokine conversion processes.[3] The importance of such transmembrane protein shed by the ADAM enzyme family has been increasingly recognized. For example, ADAM-17 was identified as a tumor necrosis factor α (TNF-α) converting enzyme (TACE) that catalyzes the processing of membrane-anchored proTNF-α to produce a soluble form[4] that contributes to a variety of inflammatory diseases.[5] Other ADAM enzymes are also thought to be involved in such important shedding processes. A recent study indicated that ADAM-9 is involved in the shedding of heparin-binding epidermal growth factor (EGF)-1ike growth factor (HB-EGF),[6] a member of the EGF family that stimulates cell growth and differentiation,[7,8] although the responsible enzyme is not known. HB-EGF has been implicated as a participant in a variety of normal and aberrant processes such as wound healing, blastocyst implantation, SMC hyperplasia, atherosclerosis, and tumor growth.[9] Therefore, specific inhibitors of ADAM enzymes are also considered to be attractive targets in drug discovery research.

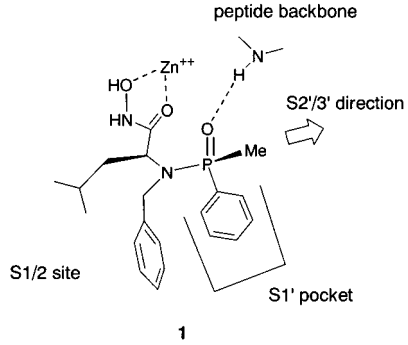

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Figure 1. Binding interaction of the phosphinamide inhibitor in MMP enzyme.

Recently extensive efforts to enhance potency and selectivity for inhibition of these metalloproteinases have been reported;[2] however, there still remains considerable scope for optimization of increasing enzyme selectivity in order to reduce side effects. In general, these studies have focused on the optimization of the P1′ portions of the inhibitors because X-ray analyses of the enzyme−inhibitor complex suggested that the S1′ pocket is a selectivity pocket for MMP inhibitors.[2,10−12] Recently, Pikul et al. reported that the phosphinamide-based hydroxamic acid **1** exhibited potent inhibitory activity against MMPs, and the binding interaction was proposed on the basis of X-ray data of the inhibitor−enzyme complex (Figure 1).[13] This type of compound has an additional chiral center at the phosphorus atom different from those of the corresponding sulfonamide derivatives, and it has been found that the stereochemistry at the phosphorus was very important for activity. In recent reports, many chiral compounds exhibited

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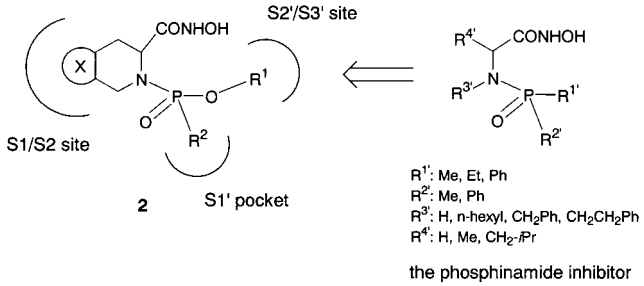
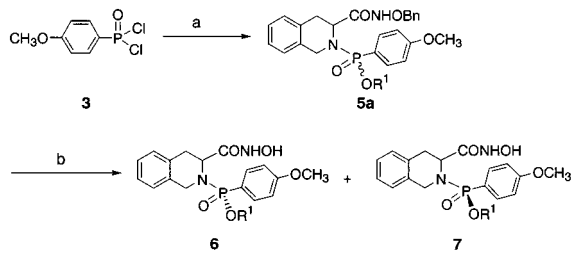


Figure 2. Strategy for the study of SAR.

Scheme 1[a]



[a] (a) (3*R*)- or (3*S*)-*N*-benzyloxy-1,2,3,4-tetrahydroisoquinoline-3-hydroxamide (**4**), then R^1OH, THF or pyridine; (b) H_2, Pd−C.

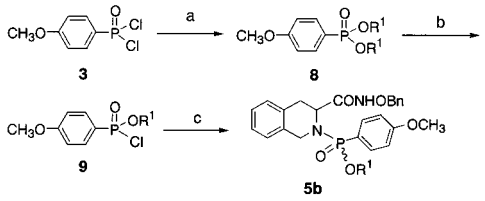
selective profiles in their inhibitions;[14−18] however, there was little information of a structure−activity relationship (SAR) regarding the effects of the chirality on enzyme selectivity. On the basis of these findings, we supposed that such a chirality might induce enzyme selectivity. Thus, we have designed the phosphonamide-based inhibitor **2** in order to study the SAR in more detail and to discover a new class of selective MP inhibitors. In our design, the ester moiety R^1 could be readily converted to various substituents compared to the corresponding moiety in the phosphinamides **1**, and this modification enables us to identify new interactions with S2′/S3′ in the enzymes (Figure 2). Moreover, the modification of R^2 and X moieties could provide not only the SAR information for MPs but structural information about the responsible enzyme for HB-EGF shedding.

In this paper, we describe the design and syntheses of novel and potent phosphonamide-based inhibitors for MP enzymes. New derivatives synthesized here were assayed for the inhibition of three types of MMPs: collagenase 1 (MMP-1), stromelysin 1 (MMP-3), and gelatinase B (MMP-9). In addition, we also tested their inhibition activitites against the ADAM family, TACE, and HB-EGF shedding. The SAR for these enzymes was discussed, and the possible binding mode in MP enzymes was also analyzed by computer modeling.

Chemistry

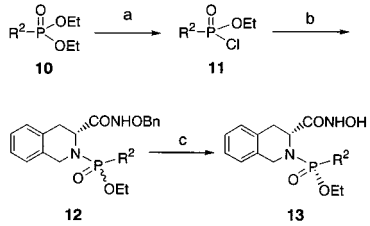
The modification of the R^1 moiety was performed by two methods (method A or B). First, the synthesis of phosphonamide derivatives by a one-pot methodology is presented in Scheme 1 (method A). Commercially available *p*-methoxyphenylphosphonic dichloride **3** was treated with (3*R*)- or (3*S*)-*N*-benzyloxy-1,2,3,4-tetrahydroisoquinole-3-carboxamide **4** (prepared from the corresponding carboxylic acid[19]) in pyridine, and then the resulting monochloride was allowed to react with the appropriate alcohol to give the phosphonamide **5a** (ca. 2:1 mixture of two diastereomers) in a one-pot reaction. In this step, if THF was used as the solvent in the presence of *N*-methylmorpholine, the diastereomeric isomers were produced in an approximately 1:1 ratio, although the yields for the reactions were decreased. Deprotection of the benzyl group in **5a** with 10% Pd−C gave the diastereomerically pure hydroxamic acids **6** and **7** that were successfully separated by HPLC purification. In some cases, these diastereomeric isomers could be purely isolated by recrystallization.

Scheme 2[a]



[a] (a) R^1OH, NaH, THF; (b) $SOCl_2$, catalyst DMF; (c) (3*R*)-**4**, diisopropylethylamine, THF.

Scheme 3[a]



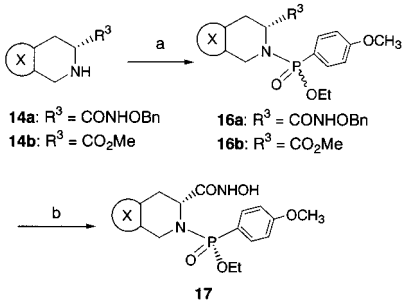
[a] (a) $SOCl_2$, catalyst DMF; (b) (3*R*)-**4**, diisopropylethylamine, THF or CH_2Cl_2; (c) H_2, Pd−C.

The stepwise synthesis of the phosphonamide was also developed as shown in Scheme 2 (method B). This methodology affords 1:1 diastereomeric products in good yields compared with the one-pot methodology (method A). Thus, *p*-methoxyphenylphosphonic dichloride **3** was treated with the appropriate sodium alkoxide to provide the diester **8**, which was converted to the corresponding phosphonyl chloride **9** in reflux with thionyl chloride in the presence of catalytic DMF. The phosphonyl chloride **9** was then coupled with (3*R*)-1,2,3,4-tetrahydroisoquinoline derivative **4** in THF in the presence of diisopropylethylamine (DIEA) to yield the (3*R*)-phosphonamide **5b** as a mixture of two diastereomers (1:1). A further step was identical to the one described above.

The general procedure for the replacement of R^2 by various groups is shown in Scheme 3 (method C). The required diethyl phosphonate **10** was prepared according to the method reported in the literature.[20] Compound **10** was refluxed in thionyl chloride in the presence of a catalytic amount of DMF to give the chlorophosphonate **11**. Compound **11** was then coupled with the (3*R*)-**4** in the presence of DIEA to give the (3*R*)-phosphonamide **12**. Completion of the synthesis was achieved as described in method A to afford the desired product **13**.

Another series of derivatives shown in Table 3 was obtained by reaction of the appropriate cyclic amine **14a** or **14b** with ethyl *p*-methoxyphenylchlorophosphonate **15**, shown in Scheme 4 (method D). The (*R*)-cyclic amine **14a** or **14b** was respectively prepared from natural or unnatural amino acid derivatives under Pictet−Spengler conditions.[21] The conversion of the R^3 moiety into hydroxamic acid was done with general methods.

Scheme 4[a]



[a] (a) 4-Methoxyphenylchlorophosphonic acid ethyl ester (**15**), diisopropylethylamine, THF; (b) NaOH, then WSC, HOBt, hydroxylamine hydrochloride or H_2, Pd−C.

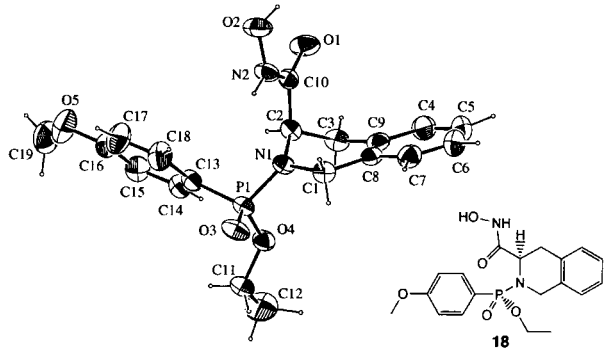


Figure 3. ORTEP view of X-ray structure of **18**.

To determine the absolute configuration at the phosphorus atom, compound **18** was crystallized and analyzed by X-ray diffraction.[22] As shown in Figure 3, the configuration at the phosphorus atom of compound **18** was determined to be *S*. For all diastereomeric pairs in this series, the configuration of phosphorus was assigned by a characteristic signal pattern in ^{1}H NMR.

All compounds synthesized here were found to be stable under assay conditions. However, decompositions of the compounds were observed under acidic conditions (below pH 4) due to the lability of P−N bonds.[23]

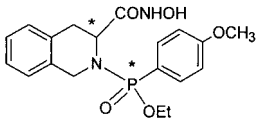
Results and Discussion

In the course of the study of the MMP inhibitor in our laboratory, we have found that conversion of an acyclic structure into a bicyclic structure resulted in the enhancement of the inhibitory activity.[24] Matter et al. also reported that rigid bicyclic inhibiors based on the 1,2,3,4-tetrahydroisoquinoline ring were potent MMPs inhibitors.[25] Therefore, we chose the 1,2,3,4-tetrahydroisoquinoline ring as the X moiety in the initial SAR study.

At first, we synthesized all four diasteromeric isomers of the ethyl ester derivative as typical phosphonamide inhibitors (**18**−**21**) and tested for the inhibition of MMP-1, -3, -9, and TACE to study the effect of the stereochemistry on the activities. Recombinant human collagenase-1 (MMP-1), stromelysin 1 (MMP-3), gelatinase B (MMP-9), and TACE were used for these assays. We also examined their abilities to inhibit HB-EGF release from fibrosarcoma HT-1080 transfectants expressing alkaline phosphatase (AP)-tagged HB-EGF stimulated by 12-*O*-tetradecanoylphorbol 13-acetate (TPA).[26] It can be seen from the results (Tables 1−4) that K_i values for MMPs and TACE inhibitions are in the nanomolar range, while IC_{50} values against HB-EGF shedding are in the micromolar range. This difference in the order of inhibition activities seems to attribute to the difference in assay system.[27] As shown in Table 1, (3*R*,*R*)-compound **19** exhibited potent inhibitory activity against all enzymes but the other isomers showed no activity or moderate activities. The importance of the *R* configuration at the α-carbon of the hydroxamate was well documented;[2,28−30] however, (3*S*,*R*)-compound **20** showed moderate activity for MMP-1 and -9, while both (3*R*,*S*)-compound **18** and (3*S*,*S*)-compound **21** showed no activity. These results suggested that the stereochemistry of the phosphorus was more important for the activity than that of α-carbon of the hydroxamate.

Because the (3*R*,*R*)-configuration was found to be very important for the activity, the SAR study for the ester group R^1 was performed with (3*R*,*R*)-phosphonamide derivatives, and the results are shown in Table 2. All of the compounds in the series exhibited significantly potent activity against MMPs with almost similar K_i values. The increase of steric bulk of the R^1 group resulted in a dramatic decrease of the inhibitory activity

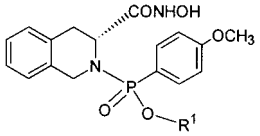
Table 1. Effects of Stereochemistry on in Vitro Activity for the Phosphonamide Derivatives



compd	stereochemistry (C-3, P)	Ki (nM)[a] MMP-1	MMP-3	MMP-9	TACE	IC_{50} (nM)[a] HB-EGF
18	(*R*,*S*)	>850	>650	>800	>780	>10 000
19	(*R*,*R*)	4.58	5.20	5.05	7.15	230
20	(*S*,*R*)	759	>650	349	>780	>10 000
21	(*S*,*S*)	>850	>650	>800	>780	>10 000

[a] See Experimental Section for details of experimental assays.

Table 2. In Vitro Profile of R^1-Modified Phosphonamide Derivatives



Compd	R^1	*Ki* (nM)[a] MMP-1	MMP-3	MMP-9	TACE	IC_{50} (nM)[a] HB-EGF
22	Me	3.64	2.95	2.03	8.92	70
19	Et	4.58	5.20	5.05	7.15	230
23[b]	*n*-butyl	5.39	24.0	9.12	nd	810
24	*n*-hexyl	10.9	5.19	6.57	32.7	1600
25[b]	Isopropyl	17.8	24.4	23.2	nd	2240
26		6.67	2.43	4.56	15.5	1340
27[b]		4.43	8.15	5.22	42.2	3110
28		7.33	12.8	6.47	90.2	3560
29	$-CH_2CH_2N(Et)_2$	10.7	82.4	16.7	33.2	1140
30		3.14	6.41	3.42	120	180
31	$-CH_2CH_2O\,CH_2CH_3$	6.75	5.43	6.36	18.9	510

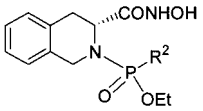
[a] See Experimental Section for details of experimental assays. [b] Diastereomeric mixture. nd, not determined.

for HB-EGF shedding (compounds **23**−**28**). The phenetyl derivative **27** and biphenylethyl derivative **28** were significantly less potent for HB-EGF, and compound **28** showed a 50-fold decrease of inhibitory activity for HB-EGF shedding compared to compound **22**. These bulky derivatives also showed a modest activity against TACE. Incorporation of a nitrogen atom into the phenyl group in compound **27** resulted in a dramatic improvement of inhibitory activity for HB-EGF (compound **30**), although this replacement led to a further decrease of the inhibitory activity against TACE. Introduction of a diethylamino group (compound **29**) resulted in a decrease of the inhibitory activity for MMP-3 and HB-EGF shedding and a modest decrease for TACE. However, no significant changes of the activities against MMP-1 and -9 were observed with these compounds. These results suggested that MMP-3 and the ADAM enzymes appear to be sensitive to the modification of the ester groups in the phosphonamides, while MMP-1 and -9 have little sensitivity to the ester groups. This sensitivity to MMP-3 was supported by the fact that the phosphinamide-based inhibitors showed similar inhibitory profiles.[13]

On the basis of the results of SAR for the ester group, the R^1 group seems to have little interaction with the S2′/S3′ site of MMPs, especially of MMP-1. Therefore, it might be useful to introduce additional properties to the inhibitors, such as water solubility, without any loss of inhibitory potencies.

The effects of the substituents R^2 attached to the phosphonamide moiety were shown in Table 3. Introduction of a long alkoxyalkyl chain at the para position of the phenyl ring resulted in a significant decrease of the inhibitory activity against MMP-1 (compound **40**), while the inhibitory activity for the other enzymes was maintained or increased compared to that of the parent compound **19**. These results suggested that the substituents R^2 would bind to the S1′ pocket of MP enzymes because this pocket is deep for most MMP enzymes but it is short for MMP-1.[10,30] On the other hand, bulky substituents at the para position of the phenyl ring increased the inhibitory activity for MMP-9 (compounds **41**−**44**). It was noteworthy that compound **42** was an extremely potent inhibitor against MMP-9, and it was at least 250-fold potent compared with the best phosphinamide-based inhibitor ($R^{1'}$ = Me; $R^{2'}$ = Ph; $R^{3'}$ = Bn; $R^{4'}$ = CH_2iPr, IC_{50} = 20.6 nM for MMP-9).[13] Insertion of an alkyl chain between the phenyl ring and the phosphonamide moiety resulted in a slight decrease of the inhibitory activity for all enzymes, but the alkenyl chain dramatically decreased the activity for MMP-1 (compounds **45** and **46**). Interestingly, the aniline derivative (compound **38**) was a moderately potent inhibitor for TACE and HB-EGF shedding, but the pyridine derivative (compound **37**) was a poor inhibitor for those enzymes. Substitution of the *p*-methoxy group in the phenyl ring with a *p*-methyl group (compound **33**) resulted in a decrease of the potency against MMP-3, -9, TACE, and HB-EGF shedding but did not affect the activity for MMP-1. The *p*-fluorophenyl derivative **34** showed strong inhibition against MMP-1 but showed modest activity for MMP-3 and -9. As a result, the selectivity for MMP-1 vs MMP-3 and -9 was increased to 66-fold and 54-fold, respectively. This result implied a possibility of a specific interaction of the fluorine atom with the residues in the S1′ pocket of MMP-1, such as a hydrogen bond. The *m*-fluorophenyl and *o*-fluorophenyl substituents decreased the inhibitory activity against MMP-1 and HB-EGF shedding (compounds **35** and **36**). These lower activities of compounds **35** and **36** can be explained not only by the narrowness of the S1′ pocket but also by the inability of the fluorine atoms to develop the putative hydrogen bonding.

Table 3. In Vitro Profile of R^2-Modified Phosphonamide Derivatives



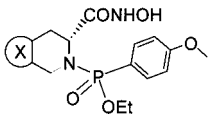
Compd	R^2	Ki (nM)[a] MMP-1	MMP-3	MMP-9	TACE	IC_{50} (nM)[a] HB-EGF
19		4.58	5.20	5.05	7.15	230
32[b]		11.4	34.9	42.7	11.2	370
33		4.90	72.9	41.8	500	1890
34		0.97	64.4	51.9	nd	300
35		27.1	51.4	80.1	22.8	830
36		24.0	47.8	32.1	73.6	1060
37		17.4	190	278	777	2600
38		58.8	162	95.6	75.8	660
39		18.7	67.0	59.2	59.6	890
40		>850	29.6	14.2	2.98	70
41		9.65	4.36	0.31	50.0	1960
42		1.81	0.53	0.08	7.89	260
43		39.8	4.85	0.60	13.8	660
44		6.50	0.50	0.10	21.7	1670
45		160	29.7	88.8	37.4	910
46		20.8	11.6	38.3	14.2	700

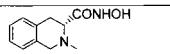
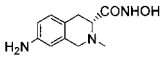
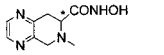
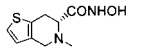
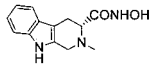
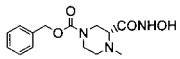
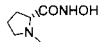
[a] See Experimental Section for details of experimental assays. [b] Methyl ester derivatives.

Replacement of 1,2,3,4-tetrahydroisoquinoline ring with other heterocycles provided insight into the structural requirements of the S1/S2 binding site (Table 4). Many of the compounds were quite potent inhibitors of all enzymes. β-Carboline derivative **50** was slightly less potent for HB-EGF shedding. It was noteworthy that reduction of ring size (proline derivative **52**) resulted in a significant loss in potency for all enzymes. This observation may imply a limit to the dihedral angle between hydroxamate and phosphonamide acceptable to these enzymes.

To better understand the observed SAR, the binding model of the **19**−MMP-3CD complex was constructed using recently published crystal structures of MMP-3 complexed with a phophinamide derivative.[31] As shown in Figures 4 and 5, the hydroxamate was tightly bound to the zinc ion and the *p*-methoxyphenyl moiety was placed in the S1′ pocket. The oxygen atom of the phosphonamide was positioned at the hydrogen bond distance with the main chain of Leu-164 and Ala-165. The ethyl ester group seems to lie along the binding

Table 4. In Vitro Profile of Phosphonamide Derivatives Modified with Various Ring



Compd	Ring structure	*Ki* (nM)[a]				IC_{50} (nM)[a]
		MMP-1	MMP-3	MMP-9	TACE	HB-EGF
19		4.58	5.20	5.05	7.15	230
47		10.7	12.3	11.0	37.4	190
48[b]		7.80	10.7	7.00	13.7	560
49		4.10	5.20	3.80	6.45	520
50[c]		2.02	2.04	1.13	2.20	800
51		20.1	14.9	5.20	7.99	430
52		>850	527	316	>780	>10000

[a] See Experimental Section for details of experimental assays. [b] Synthesized from (+)-form of the corresponding cyclic amine **14a**, although the configuration at C-7 was not established. [c] Methyl ester derivatives.

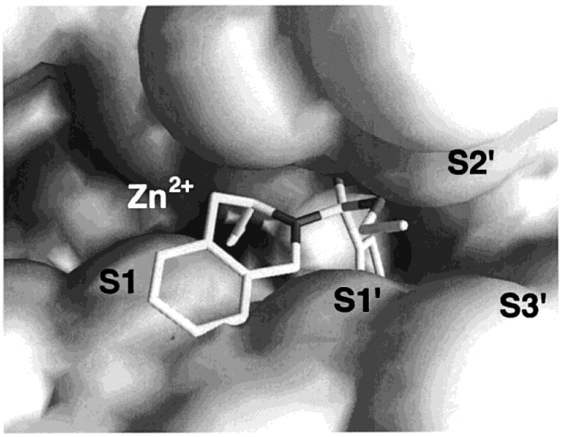


Figure 4. Docking model of MMP-3−**19** complex.

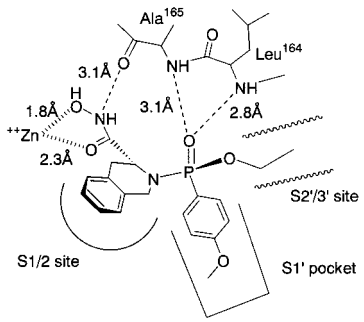


Figure 5. Expected binding mode of **19** in MMP-3.

groove of substrate backbone and to produce a van der Waals interaction. The fact that steric bulk ester derivatives (compounds **23**−**28**) dramatically decreased the potency for HB-EGF shedding suggested that the corresponding groove in the responsible enzyme for HB-EGF shedding might be narrow compared to the other MP enzymes. The tetrahydroisoquinoline ring was stacked on a S1/S2 site and also appears to produce a van der Waals interaction. This modeling study strongly supported the observed results that the *R*-isomer at the phosphorus center was only active, and the *S*-isomer showed no potency because of the conflict with the residues (Leu-164 and Ala-165) of MMP-3.

Conclusions

The design and synthesis of a new generation of metalloproteinases inhibitors have been described. Among the four diastereomeric isomers **18**−**21**, compound **19** having an *R*-configuration both at the α-carbon of hydroxamate and at the phosphorus atom was found to be a potent inhibitor for all MP enzymes, while the other diastereomeric isomers were almost inactive. We have synthesized a number of (*R*,*R*)-derivatives modified in each three portions, R^1, R^2, and X. Evaluation of these compounds in in vitro assays showed that most of compounds were potently active against all enzymes. For the modification of the R^1 group, we showed that compound **28** with a biphenyl group was surprisingly less potent against HB-EGF shedding compared to compound **22**. Introduction of long alkoxyalkyl chains for the R^2 group resulted in a significant decrease of the inhibitory activity for MMP-1 (compound **40**), while the activity for the other enzymes was maintained. In contrast, bulky substituents for the R^2 group increased the inhibitory activity for MMP-9 (compounds **41**−**44**). Regarding the SAR for the X moiety, reduction of the ring size (proline derivative **52**) resulted in a significant decrease of the inhibitory activity against all enzymes. Subsequent modeling work suggested that key interactions of enzyme and inhibitor provided the explanation for the observed stereoselectivity. These results revealed the potential of phosphonamide derivatives as a new type of candidate for metalloproteinase inhibitors.

Experimental Section

1. General Methods and Materials. All commercially available starting materials and solvents were reagent grade. Melting points were uncorrected. ^{1}H NMR spectra were measured at 250 MHz on a Bruker DPX-250 using $CDCl_3$ or DMSO-d_6 as the solvent. Mass spectra were determined on a Perceptive Biosystems Voyager-DE RP spectrometer. Elemental analyses were performed by Sumika Chemical Analysis Service, Ltd., Japan, and the results are within ±0.4% of the calculated values unless otherwise noted.

(3*R*)-2-*tert*-Butoxycarbonyl-1,2,3,4-tetrahydroisoquinole-3-carboxylic Acid. To a solution of (3*R*)-1,2,3,4-tetrahydroisoquinole-3-carboxylic acid hydrochlorde[19] (17.1 g, 80 mmol) in 200 mL of 50% aqueous 1,4-dioxane was added sodium carbonate (17.0 g, 160 mmol) and $(Boc)_2O$ (21.0 g, 96 mmol), and the mixture was stirred at room temperature overnight. The reaction mixture was concentrated to a half-volume in vacuo, and the solution was acidified with 1 N HCl (pH = 3). The solution was extracted with AcOEt, and the organic layer was washed successively with 1 N HCl and brine and dried over $MgSO_4$. The solvent was evaporated to give the title compound (13.5 g, 61%): ^{1}H NMR (DMSO-d_6) δ 1.37 (s, 4.5H), 1.44 (s, 4.5H), 3.00−3.25 (m, 2H), 4.35−4.60 (m, 2H), 4.60−4.70 (m, 0.5H), 4.80−4.90 (m, 0.5H), 7.15−7.25 (m, 4H).

(3*R*)-*N*-Benzyloxy-2-*tert*-butoxycarbonyl-1,2,3,4-tetrahydroisoquinole-3-carboxamide. To a solution of (3*R*)-2-*tert*-butoxycarbonyl-1,2,3,4-tetrahydroisoquinole-3-carboxylic acid (13.5 g, 48.7 mmol) in DMF (200 mL) was added WSC (10.4 g, 54 mmol), HOBt (8.3 g, 54 mmol), *O*-benzylhydroxylamine

hydrochloride (8.6 g, 54 mmol), and triethylamine (5.5 g, 54 mmol), and the mixture was stirred at room temperature overnight. The reaction mixture was diluted with AcOEt (200 mL), washed successively with 1 N HCl, saturated $NaHCO_3$, and brine, and dried over $MgSO_4$. The solvent was evaporated, and the residue was recrystallized from AcOEt/hexane to give the title compound (7.6 g, 41%): ^{1}H NMR ($CDCl_3$) δ 1.41 (s, 4.5H), 1.52 (s, 4.5H), 3.15−3.35 (m, 2H), 4.45−4.75 (m, 2H), 4.80−4.85 (m, 0.5H), 5.00−5.20 (m, 2H), 5.15−5.25 (m, 0.5H), 7.05−7.40 (m, 9H).

(3*R*)-*N*-Benzyloxy-1,2,3,4-tetrahydroisoquinole-3-carboxamide (4). (3*R*)-*N*-benzyloxy-2-*tert*-butoxycarbonyl-1,2,3,4-tetrahydroisoquinole-3-carboxamide (7.6 g, 19.9 mmol) was dissolved in 4 N HCl/AcOEt (50 mL), and the solution was stirred at room temperature for 2 h. The precipitates were filtered and washed with Et_2O. The solids were dissolved in water, neutralized with $NaHCO_3$, and extracted with AcOEt. The organic layer was washed with brine and dried over $MgSO_4$. Removal of the solvent gave the title compound (4.6 g): ^{1}H NMR (DMSO-d_6) δ 2.70−2.80 (m, 2H), 3.30−3.35 (m, 1H), 3.75−3.95 (m, 2H), 4.80 (s, 2H), 6.95−7.15 (m, 4H), 7.30−7.45 (m, 5H).

(3*S*)-*N*-Benzyloxy-1,2,3,4-tetrahydroisoquinole-3-carboxamide was synthesized from (3*S*)-1,2,3,4-tetrahydroisoquinole-3-carboxylic acid hydrochlorde[19] utilizing smilar reaction conditions.

General Procedure for Preparing Phosphonamide Derivatives (Method A). Synthesis of (3*R*)-*N*-Hydroxy-2-[(*R* and *S*)-ethoxy(4-methoxyphenyl)phosphoryl]-1,2,3,4-tetrahydroisoquinoline-3-carboxamide (18 and 19). Diastereomers of (3*R*)-*N*-Benzyloxy-2-[(*RS*)-ethoxy(4-methoxyphenyl)phosphoryl]-1,2,3,4-tetrahydroisoquinoline-3-carboxamide (5a). To a solution of 4-methoxyphenylphosphonic dichloride (398 mg, 1.77 mmol) in 5 mL of pyridine was added (3*R*)-*N*-benzyloxy-1,2,3,4-tetrahydroisoquinole-3-carboxamide **4** (500 mg, 1.77 mmol, prepared from (3*R*)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid[19] and *O*-benzylhydroxylamine), and the mixture was stirred at room temperature for 15 min under an argon atmosphere. Then, ethanol (0.1 mL, 1.77 mmol) was added to the solution, and the mixture was stirred at room temperature for 1 h. The reaction mixture was concentrated in vacuo, and the remaining pyridine was completely removed by azeotropic evaporation with toluene. The residual oil was diluted with AcOEt, and the solution was washed successively with saturated $NaHCO_3$ and brine and dried over $MgSO_4$. The solvent was evaporated, and the residue was purified by column chromatography on silica gel, eluting with AcOEt to give the title compound as a diasteromeric mixture (ca. 2:1, 466 mg, 55%): ^{1}H NMR ($CDCl_3$) δ 1.19 (t, J = 7.1 Hz, 1H), 1.31 (t, J = 7.1 Hz, 2H), 2.75−2.95 (m, 1H), 3.30−3.45 (m, 1H), 3.81 (s, 2H), 3.85 (s, 1H), 3.90−4.30 (m, 4H), 4.45−4.55 (m, 0.3 H), 4.75−4.85 (m, 2H), 6.85−6.95 (m, 3H), 7.05−7.20 (m, 3H), 7.25−7.35 (m, 5H), 7.45−7.70 (m, 2H), 9.71 (br s, 0.3H), 10.11 (br s, 0.7H).

(3*R*)-*N*-Hydroxy-2-[(*R* and *S*)-ethoxy(4-methoxyphenyl)phosphoryl]-1,2,3,4-tetrahydroisoquinoline-3-carboxamide (18 and 19). A mixture of compound **5** (466 mg, 0.97 mmol) and 10% Pd−C (40 wt %, prewashed with EtOH) in EtOH (20 mL) was stirred at room temperature under H_2 for 3 h. Pd−C was filtered off, and the filtrate was concentrated. The residue was purified by HPLC (YMC-ODS, CH_3CN/water 30:70) to afford two diastereomerically pure hydroxamic acid. **19** (80 mg, 21%) was eluted first, and then **18** (127 mg, 34%) was eluted.

(3*R*)-*N*-Hydroxy-2-[(*S*)-ethoxy(4-methoxyphenyl)phosphoryl]-1,2,3,4-tetrahydroisoquinoline-3-carboxamide (18): Recrystallization from EtOH/CH_3CN gave colorless crystals; mp 165−166 °C; ^{1}H NMR (DMSO-d_6) δ 1.31 (t, J = 7.0 Hz, 3H), 2.90−3.10 (m, 2H), 3.75 (s, 3H), 3.90−4.20 (m, 3H), 4.40−4.60 (m, 2H), 6.90−7.10 (m, 6H), 7.54 (dd, J = 8.8 and 12.3 Hz, 2H), 8.76 (br s, 1H), 10.54 (br s, 1H); MALDI-TOF MS m/z 429 [M + K]$^+$, 413 [M + Na]$^+$, 391 [M + H]$^+$. Anal. ($C_{19}H_{23}N_2O_5P$) C, H, N.

(3*R*)-*N*-Hydroxy-2-[(*R*)-ethoxy(4-methoxyphenyl)phosphoryl]-1,2,3,4-tetrahydroisoquinoline-3-carboxamide (19): colorless solids; ^{1}H NMR (DMSO-d_6) δ 1.14 (t, J = 7.0 Hz, 3H), 2.90−3.05 (m, 2H), 3.65−3.80 (m, 1H), 3.79 (s, 3H), 3.85−4.00 (m, 1H), 4.14 (dd, J = 7.6 and 16.1 Hz, 1H), 4.30 (dd, J = 4.5 and 16.1 Hz, 1H), 4.35−4.45 (m, 1H), 7.02 (dd, J = 3.1 and 8.9 Hz, 2H), 7.00−7.15 (m, 4H), 7.69 (dd, J = 8.8 and 12.4 Hz, 2H), 8.78 (br s, 1H), 10.57 (br s, 1H); MALDI-TOF MS m/z 429 [M + K]$^+$, 413 [M + Na]$^+$, 391 [M + H]$^+$. Anal. ($C_{19}H_{23}N_2O_5P$) C, H, N. Calcd: C, 58.46; H, 5.94; N, 7.18. Found: C, 58.00; H, 6.14; N, 7.14.

Compounds **20** and **21** were synthesized from (3*S*)-*N*-benzyloxy-1,2,3,4-tetrahydroisoquinole-3-carboxamide utilizing similar reaction conditions.

(3*S*)-*N*-Hydroxy-2-[(*R*)-ethoxy(4-methoxyphenyl)phosphoryl]-1,2,3,4-tetrahydroisoquinoline-3-carboxamide (20): colorless solids; 23% yield; ^{1}H NMR (DMSO-d_6) δ 1.16 (t, J = 7.0 Hz, 3H), 2.90−3.05 (m, 2H), 3.70−3.90 (m, 1H), 3.81 (s, 3H), 3.90−4.05 (m, 1H), 4.16 (dd, J = 7.6 and 16.0 Hz, 1H), 4.32 (dd, J = 4.9 and 16.0 Hz, 1H), 4.40−4.50 (m, 1H), 7.00−7.20 (m, 6H), 7.72 (dd, J = 8.8 and 12.4 Hz, 2H), 8.80 (br s, 1H), 10.60 (br s, 1H); MALDI-TOF MS m/z 429 [M + K]$^+$, 413 [M + Na]$^+$, 391 [M + H]$^+$. Anal. ($C_{19}H_{23}N_2O_5P$) C, H, N.

(3*S*)-*N*-Hydroxy-2-[(*S*)-ethoxy(4-methoxyphenyl)phosphoryl]-1,2,3,4-tetrahydroisoquinoline-3-carboxamide (21): colorless solids; 25% yield; ^{1}H NMR (DMSO-d_6) δ 1.32 (t, J = 7.0 Hz, 3H), 2.90−3.15 (m, 2H), 3.76 (s, 3H), 3.95−4.20 (m, 3H), 4.40−4.60 (m, 2H), 6.90−7.10 (m, 6H), 7.56 (dd, J = 8.8 and 12.3 Hz, 2H), 8.77 (br s, 1H), 10.56 (br s, 1H); MALDI-TOF MS m/z 429 [M + K]$^+$, 413 [M + Na]$^+$, 391 [M + H]$^+$. Anal. ($C_{19}H_{23}N_2O_5P$) C, H, N.

Compounds **22**−**24**, **26**−**30** were also synthesized using a procedure similar to the procedure for the preparation of **19**.

(3*R*)-*N*-Hydroxy-2-[(*R*)-methoxy(4-methoxyphenyl)-phosphoryl]-1,2,3,4-tetrahydroisoquinoline-3-carboxamide (22): colorless solids; 12% yield; ^{1}H NMR (DMSO-d_6) δ 2.80−3.05 (m, 2H), 3.48 (d, J = 11.1 Hz, 3H), 3.80 (s, 3H), 4.14 (dd, J = 7.5 and 16.1 Hz, 1H), 4.30 (dd, J = 4.9 and 16.1 Hz, 1H), 4.35−4.45 (m, 1H), 7.00−7.15 (m, 6H), 7.69 (dd, J = 8.9 and 12.4 Hz, 2H), 8.79 (br s, 1H), 10.58 (br s, 1H); MALDI-TOF MS m/z 415 [M + K]$^+$, 399 [M + Na]$^+$, 377 [M + H]$^+$. Anal. ($C_{18}H_{21}N_2O_5P$) C, H, N.

(3*R*)-*N*-Hydroxy-2-[(*RS*)-*n*-butoxy(4-methoxyphenyl)-phosphoryl]-1,2,3,4-tetrahydroisoquinoline-3-carboxamide (23): a mixture of two diastereomers; 60% yield; ^{1}H NMR (DMSO-d_6) δ 0.79 (t, J = 7.3 Hz, 1.2H), 0.93 (t, J = 7.3 Hz, 1.8H), 1.10−1.60 (m, 2.8H), 1.60−1.75 (m, 1.2H), 2.80−3.20 (m, 2H), 3.76 (s, 1.8H), 3.81 (s, 1.2H), 3.80−4.60 (m, 5H), 6.85−7.20 (m, 6H), 7.55 (dd, J = 8.8 and 12.3 Hz, 1.2H), 7.72 (dd, J = 8.8 and 12.4 Hz, 0.8H), 8.76 (s, 0.6H), 8.79 (s, 0.4H), 10.57 (s, 1H); MALDI-TOF MS m/z 457 [M + K]$^+$, 441 [M + Na]$^+$, 419 [M + H]$^+$. Anal. ($C_{21}H_{27}N_2O_5P$) C, H, N.

(3*R*)-*N*-Hydroxy-2-[(*R*)-*n*-hexyloxy(4-methoxyphenyl)-phosphoryl]-1,2,3,4-tetrahydroisoquinoline-3-carboxamide (24): colorless solids; 8% yield; ^{1}H NMR (DMSO-d_6) δ 0.78 (t, J = 6.6 Hz, 3H), 1.00−1.30 (m, 6H), 1.45−1.55 (m, 2H), 2.85−3.05 (m, 2H), 3.60−3.70 (m, 1H), 3.80 (s, 3H), 3.80−3.90 (m, 1H), 4.08 (dd, J = 7.9 and 16.1 Hz, 1H), 4.29 (dd, J = 4.8 and 16.1 Hz, 1H), 4.40−4.50 (m, 1H), 7.03 (dd, J = 3.0 and 8.7 Hz, 2H), 7.00−7.15 (m, 4H), 7.70 (dd, J = 8.7 and 12.4 Hz, 2H), 8.77 (br s, 1H), 10.58 (br s, 1H); MALDI-TOF MS m/z 485 [M + K]$^+$, 469 [M + Na]$^+$, 447 [M + H]$^+$. Anal. ($C_{23}H_{31}N_2O_5P$) C, H, N.

(3*R*)-*N*-Hydroxy-2-[(*R*)-cyclohexylmethyloxy(4-methoxyphenyl)phosphoryl]-1,2,3,4-tetrahydroisoquinoline-3-carboxamide (26): colorless solids; 13% yield; ^{1}H NMR (DMSO-d_6) δ 0.75−1.00 (m, 2H), 1.00−1.20 (m, 3H), 1.30−1.70 (m, 6H), 2.80−3.10 (m, 2H), 3.40−3.50 (m, 1H), 3.60−3.75 (m, 1H), 3.81 (s, 3H), 4.09 (dd, J = 7.8 and 16.2 Hz, 1H), 4.30 (dd, J = 5.1 and 16.2 Hz, 1H), 4.40−4.50 (m, 1H), 7.00−7.15 (m, 6H), 7.72 (dd, J = 8.7 and 12.4 Hz, 2H), 8.79 (s, 1H), 10.58 (s, 1H); MALDI-TOF MS m/z 497 [M + K]$^+$, 481 [M + Na]$^+$, 459 [M + H]$^+$. Anal. ($C_{24}H_{31}N_2O_5P$) C, H, N.

(3*R*)-*N*-Hydroxy-2-[(*RS*)-phenethyloxy(4-methoxyphenyl)-phosphoryl]-1,2,3,4-tetrahydroisoquinoline-3-carboxamide (27): a mixture of two diastereomers; 8% yield; ^{1}H NMR (DMSO-d_6) δ 2.83 (t, J = 7.2 Hz, 1H), 2.90−3.05 (m, 3H), 3.74 (s, 1.5H), 3.80 (s, 1.5H), 3.80−4.55 (m, 5H), 6.80−7.35 (m, 11H), 7.45 (dd, J = 8.7 and 12.2 Hz, 1H), 7.63 (dd, J = 8.7 and 12.5 Hz, 1H), 8.75 (br s, 0.5H), 8.78 (br s, 0.5H), 10.53 (br s, 0.5H), 10.57 (br s, 0.5H); MALDI-TOF MS *m*/*z* 505 [M + K]$^+$, 489 [M + Na]$^+$, 467 [M + H]$^+$. Anal. ($C_{25}H_{27}N_2O_5P$) C, H, N.

(3*R*)-*N*-Hydroxy-2-[(*R*)-(2-biphenyl-4-yl-ethyloxy)(4-methoxyphenyl)phosphoryl]-1,2,3,4-tetrahydroisoquinoline-3-carboxamide (28): colorless solids; 20% yield; ^{1}H NMR (DMSO-d_6) δ 2.80−3.00 (m, 4H), 3.79 (s, 3H), 3.80−4.20 (m, 3H), 4.20−4.30 (m, 1H), 4.40−4.50 (m, 1H), 6.90−7.10 (m, 3H), 7.00−7.10 (m, 3H), 7.21 (d, J = 8.3 Hz, 2H), 7.30−7.40 (m, 1H), 7.44 (t, J = 7.2 Hz, 2H), 7.52 (d, J = 9.0 Hz, 2H), 7.60−7.70 (m, 4H), 8.78 (br s, 1H), 10.59 (br s, 1H); MALDI-TOF MS *m*/*z* 581 [M + K]$^+$, 565 [M + Na]$^+$, 543 [M + H]$^+$. Anal. ($C_{31}H_{31}N_2O_5P$) C, H, N.

(3*R*)-*N*-Hydroxy-2-[(*R*)-(2-diethylaminoethyloxy)(4-methoxyphenyl)phosphoryl]-1,2,3,4-tetrahydroisoquinoline-3-carboxamide (29): colorless solids; 6% yield; ^{1}H NMR (DMSO-d_6) δ 1.14 (t, J = 7.4 Hz, 3H), 1.16 (t J = 7.1 Hz, 3H), 2.95−3.00 (m, 2H), 3.05−3.20 (m, 4H), 3.30−3.40 (m, 2H), 3.81 (s, 3H), 4.00−4.10 (m, 1H), 4.20−4.40 (m, 4H), 7.00−7.15 (m, 6H), 7.76 (dd, J = 8.8 and 12.8 Hz, 2H), 8.84 (br s, 1H), 10.64 (br s, 1H); MALDI TOF MS *m*/*z* 500 [M + K]$^+$, 484 [M + Na]$^+$, 462 [M + H]$^+$. Anal. ($C_{23}H_{32}N_3O_5P$) C, H, N.

(3*R*)-*N*-Hydroxy-2-{(*R*)-[2-(pyridine-2-yl)ethyloxy](4-methoxyphenyl)phosphoryl}-1,2,3,4-tetrahydroisoquinoline-3-carboxamide (30): pale-yellow solids; 9% yield; ^{1}H NMR (DMSO-d_6) δ 2.90−3.05 (m, 4H), 3.78 (s, 3H), 3.90−4.10 (m, 2H), 4.15−4.25 (m, 2H), 4.40−4.45 (m, 1H), 6.95−7.25 (m, 9H), 7.55−7.70 (m, 3H), 8.35−8.40 (m, 1H), 8.78 (br s, 1H), 10.58 (br s, 1H); MALDI TOF MS *m*/*z* 506 [M + K]$^+$, 490 [M + Na]$^+$, 468 [M + H]$^+$. Anal. ($C_{24}H_{26}N_3O_5P$) C, H, N.

Compound **32** was synthesized from commercially available phenylphosphonic dichloride and methanol using a procedure similar to the procedure for the preparation of **19**.

(3*R*)-*N*-Hydroxy-2-[(*R*)-methoxy(phenyl)phosphoryl]-1,2,3,4-tetrahydroisoquinoline-3-carboxamide (32): colorless solids; 22% yield; ^{1}H NMR (DMSO-d_6) δ 2.85−3.05 (m, 2H), 3.51 (d, J = 11.1 Hz, 3H), 4.18 (dd, J = 7.5 and 16.2 Hz, 1H), 4.25−4.45 (m, 2H), 7.05−7.15 (m, 4H), 7.45−7.60 (m, 3H), 7.70−7.80 (m, 2H), 8.80 (br s, 1H), 10.61 (br s, 1H); MALDI-TOF MS *m*/*z* 385 [M + K]$^+$, 369 [M + Na]$^+$, 347 [M + H]$^+$. Anal. ($C_{17}H_{19}N_2O_4P$) C, H, N.

Compound **50** was synthesized from (3*R*)-*N*-benzyloxy-1,3,4,9-tetrahydro-β-carboline-3-carboxamide and methanol using a procedure similar to the procedure for the preparation of **19**.

(3*R*)-*N*-Hydroxy-2-[(*R*)-methoxy(4-methoxyphenyl)phosphoryl]-1,3,4,9-tetrahydro-β-carboline-3-carboxamide (50): colorless solids; 5% yield; ^{1}H NMR (DMSO-d_6) δ 2.80 (dd, J = 5.7 and 15.6 Hz, 1H), 3.02 (d, J = 15.6 Hz, 1H), 3.55 (d, J = 11.2 Hz, 3H), 3.80 (s, 3H), 4.22 (dd, J = 8.2 and 15.0 Hz, 1H), 4.43 (d, J = 15.0 Hz, 1H), 4.57 (dd, J = 5.7 and 8.2 Hz, 1H), 6.90−7.10 (m, 4H), 7.25 (d, J = 7.7 Hz, 1H), 7.33 (d, J = 7.4 Hz, 1H), 7.73 (dd, J = 8.8 and 12.4 Hz, 2H), 8.70 (br s, 1H), 10.58 (br s, 1H), 10.70 (br s, 1H); MALDI-TOF MS *m*/*z* 454 [M + K]$^+$, 438 [M + Na]$^+$, 416 [M + H]$^+$. Anal. ($C_{20}H_{22}N_3O_5P$) C, H, N.

Alternative Procedure for Preparing Phosphonamide Derivatives (Method B). Synthesis of (3*R*)-*N*-Hydroxy-2-[(*R*)−(2-ethoxyethyloxy)(4-methoxyphenyl)phosphoryl]-1,2,3,4-tetrahydroisoquinoline-3-carboxamide (31). (4-Methoxyphenyl)phosphonic Acid Bis(2-ethoxyethyl) Ester (8). To a solution of 2-ethoxyethanol (440 mg, 4.89 mmol) in dry THF (50 mL) was added NaH (130 mg, 5.4 mmol), and the mixture was stirred at room temperature under an argon atmosphere for 20 min. Then, 4-methoxyphenylphosphonic dichloride (500 mg, 2.22 mmol) was added to the mixture, and the stirring was continued overnight. The reaction mixture was quenched with 1 N HCl (50 mL) and extracted with AcOEt. The organic layer was washed with saturated $NaHCO_3$ and brine and dried over $MgSO_4$. The solvent was evaporated to give the diester **8** as a yellow oil (532 mg, 72%): ^{1}H NMR ($CDCl_3$) δ 1.18 (t, J = 7.0 Hz, 6H), 3.49 (t, J = 7.0 Hz, 4H), 3.60−3.70 (m, 4H), 3.85 (s, 3H), 4.10−4.25 (m, 4H), 6.95 (dd, J = 3.5 and 8.9 Hz, 2H), 7.78 (dd, J = 8.9 and 12.9 Hz, 2H).

Diastereomers of (3*R*)-*N*-benzyloxy-2-[(*RS*)-(2-ethoxyethyloxy)(4-methoxyphenyl)phosphoryl]-1,2,3,4-tetrahydroisoquinoline-3-carboxamide (5b). A mixture of (4-methoxyphenyl)phosphonic acid bis(2-ethoxyethyl) ester **8** (530 mg, 1.59 mmol) and a catalytic amount of DMF (a few drops) in thionyl chloride (5 mL) was refluxed for 3 h. The reaction mixture was concentrated in vacuo, and the residual thionyl chloride was completely removed by azeotropic evaporation with toluene to afford 469 mg of crude monochloride **9**, which was used for the next reaction without further purification. To a solution of the above product **9** in 5 mL of dry THF was added (3*R*)-*N*-benzyloxy-1,2,3,4-tetrahydroisoquinole-3-carboxamide **4** (300 mg, 1.06 mmol) and diisopropylethylamine (363 μL, 2.1 mmol), and the mixture was stirred at room temperature under an argon atmosphere overnight. The reaction mixture was concentrated, and the residual oil was diluted with AcOEt. The solution was washed successively with water, saturated $NaHCO_3$, and brine and dried over $MgSO_4$. The solvent was evaporated, and the residue was purified by column chromatography on silica gel, eluting with AcOEt to give the title compound **5b** as a diasteromeric mixture (1:1, 435 mg, 78%): ^{1}H NMR ($CDCl_3$) δ 1.17 (t, J = 7.0 Hz, 1.5H), 1.18 (t, J = 7.0 Hz, 1.5H), 2.90−3.65 (m, 6H), 3.80 (s, 1.5H), 3.85 (s, 1.5H), 3.90−4.20 (m, 4H), 4.35−4.45 (m, 0.5 H), 4.55−4.65 (m, 0.5H), 4.75−4.85 (m, 2H), 6.80−7.65 (m, 13H), 9.91 (br s, 0.5H), 10.24 (br s, 0.5H).

(3*R*)-*N*-Hydroxy-2-[(*R*)-(2-ethoxyethyloxy)(4-methoxyphenyl)phosphoryl]-1,2,3,4-tetrahydroisoquinoline-3-carboxamide (31). The title compound was prepared from **5b** following the precedure described for compound **19**: colorless solids; 31% yield; ^{1}H NMR (DMSO-d_6) δ 1.00 (t, J = 7.0 Hz, 3H), 2.90−3.20 (m, 2H), 3.30−3.40 (m, 2H), 3.45−3.55 (m, 2H), 3.80 (s, 3H), 3.75−3.90 (m, 1H), 3.90−4.05 (m, 1H), 4.10−4.35 (m, 2H), 4.45−4.50 (m, 1H), 7.00−7.15 (m, 6H), 7.72 (dd, J = 8.8 and 12.5 Hz, 2H), 8.77 (br s, 1H), 10.57 (br s, 1H); MALDI-TOF MS *m*/*z* 473 [M + K]$^+$, 457 [M + Na]$^+$, 435 [M + H]$^+$. Anal. ($C_{21}H_{27}N_2O_6P\cdot 0.5H_2O$) C, H, N.

Compounds **25** were also synthesized using a procedure similar to the procedure for the preparation of **31**.

(3*R*)-*N*-Hydroxy-2-[(*R*)-isopropyloxy(4-methoxyphenyl)-phosphoryl]-1,2,3,4-tetrahydroisoquinoline-3-carboxamide (25): a mixture of two diastereomers; 56% yield; ^{1}H NMR (DMSO-d_6) δ 1.03 (d, J = 6.1 Hz, 1.5H), 1.27 (d, J = 6.2 Hz, 1.5H), 1.31 (d, J = 6.2 Hz, 1.5H), 1.34 (d, J = 6.2 Hz, 1.5H), 2.90−3.15 (m, 2H), 3.75 (s, 1.5H), 3.81 (s, 1.5H), 3.90−4.20 (m, 1H), 4.32 (dd, J = 4.4 and 16.2 Hz, 0.5H), 4.40−4.60 (m, 2H), 4.70−4.80 (m, 0.5H), 6.85−7.20 (m, 6H), 7.53 (dd, J = 8.7 and 12.3 Hz, 1H), 7.73 (dd, J = 8.7 and 12.5 Hz, 1H), 8.77 (s, 0.5H), 8.78 (s, 0.5H), 10.58 (s, 1H); MALDI-TOF MS *m*/*z* 443 [M + K]$^+$, 427 [M + Na]$^+$, 405 [M + H]$^+$. Anal. ($C_{20}H_{25}N_2O_5P$) C, H, N.

General Procedure for Preparing Phosphonamide Derivatives (Method C). Synthesis of (3*R*)-*N*-Hydroxy-2-[(*R*)-ethoxy(4-phenoxyphenyl)phosphoryl]-1,2,3,4-tetrahydroisoquinoline-3-carboxamide (42). 4-Phenoxyphenylphosphonic Acid Diethyl Ester (10). A mixture of diethyl phosphite (2.50 g, 18.1 mmol), triethylamine (2.50 mL, 18.1 mmol), 4-bromodiphenyl ether (4.10 g, 16.5 mmol), and tetrakis(triphenylphosphine)palladium (0.82 g) was stirred at 90 °C under an argon atmosphere overnight, then cooled to room temperature. Diethyl ether was added to the reaction mixture, and the precipitate was filtered off. The filtrate was concentrated in vacuo, and the residue was purified by column chromatography on silica gel, eluting with a gradient of *n*-hexane/AcOEt 3:2 to AcOEt to give the title compound as a brown oil (5.09 g, 100%): ^{1}H NMR ($CDCl_3$) δ 1.34 (t, J = 7.0

Hz, 6H), 4.00−4.30 (m, 4H), 7.00−7.15 (m, 4H), 7.15−7.30 (m, 1H), 7.35−7.50 (m, 2H), 7.70−7.85 (m, 2H).

Diastereomers of (3*R*)-*N*-Benzyloxy-2-[(*R*)-ethoxy(4-phenoxyphenyl)phosphoryl]-1,2,3,4-tetrahydroisoquinoline-3-carboxamide (12). A mixture of 4-phenoxyphenylphosphonic acid diethyl ester **10** (5.0 g, 16.3 mmol) and a catalytic amount of DMF (a few drops) in thionyl chloride (5 mL) was refluxed for 1.5 h. The reaction mixture was concentrated in vacuo, and the residual thionyl chloride was completely removed by azeotropic evaporation with toluene to afford 4.5 g of monochloride **11**, which was used for the next reaction without further purification. To a solution of the above product **11** (180 mg, 0.60 mmol) in 2 mL of dry CH_2Cl_2 was added (3*R*)-*N*-benzyloxy-1,2,3,4-tetrahydroisoquinole-3-carboxamide 4 (170 mg, 0.60 mmol) and diisopropylethylamine (150 mg, 1.16 mmol), and the mixture was stirred at room temperature under an argon atmosphere overnight. The reaction mixture was concentrated, and the residual oil was diluted with AcOEt. The solution was washed successively with water, saturated $NaHCO_3$, and brine and dried over $MgSO_4$. The solvent was evaporated, and the residue was purified by column chromatography on silica gel, eluting with AcOEt to give the title compound **12** as a diasteromeric mixture (1:1, 140 mg, 47%): ^{1}H NMR ($CDCl_3$) δ 1.10−1.40 (m, 3H), 2.70−3.00 (m, 1H), 3.25−3.40 (m, 1H), 3.70−4.20 (m, 4H), 4.25−4.60 (m, 1H), 4.75−4.90 (m, 2H), 6.80−7.70 (m, 18H), 9.70 (s, 0.5H), 10.07 (s, 0.5H).

(3*R*)-*N*-Hydroxy-2-[(*R*)-ethoxy(4-phenoxyphenyl)phosphoryl]-1,2,3,4-tetrahydroisoquinoline-3-carboxamide (42). The title compound was prepared from **12** following the precedure described for compound **19**: yield 14%; ^{1}H NMR (DMSO-d_6) δ 1.15 (t, J = 6.9 Hz, 3H), 2.85−3.10 (m, 2H), 3.65−4.00 (m, 2H), 4.10−4.50 (m, 3H), 7.00−7.30 (m, 9H), 7.35−7.50 (m, 2H), 7.70−7.85 (m, 2H), 8.80 (s, 1H), 10.60 (s, 1H); MALDI-TOF MS *m*/*z* 491 [M + K]$^+$, 475 [M + Na]$^+$, 453 [M + H]$^+$. Anal. ($C_{24}H_{25}N_2O_5P\cdot 0.66H_2O$) C, H, N.

Compounds **33**−**38, 40, 41**, **43**, **44**, **46** were also synthesized using a procedure similar to the procedure for the preparation of **42**. Compounds **39** and **45** were synthesized from the corresponding methyl ester derivative instead of the benzyloxyamide derivative using a procedure similar to the procedure for the preparation of **12**. The obtained methyl esters required base hydrolysis and amidation to obtain the corresponding hydroxamic acids **39** and **45**, using a procedure similar to procedure for the preparation of compound **48**.

(3*R*)-*N*-Hydroxy-2-[(*R*)-ethoxy(4-methylphenyl)phosphoryl]-1,2,3,4-tetrahydroisoquinoline-3-carboxamide (33): colorless solids; 32% yield; ^{1}H NMR ($CDCl_3$) δ 1.22 (t, J = 7.1 Hz, 3H), 2.41 (s, 3H), 2.99 (dd, J = 6.5 and 16.0 Hz, 1H), 3.32 (dd, J = 4.0 and 16.0 Hz, 1H), 3.75−4.25 (m, 4H), 4.55−4.70 (m, 1H), 6.93 (d, J = 6.8 Hz, 1H), 7.05−7.35 (m, 5H), 7.55 (br s, 1H), 7.63 (d, J = 13.0 Hz, 1H), 7.68 (d, J = 13.0 Hz, 1H), 10.36 (br s, 1H); MALDI-TOF MS *m*/*z* 413 [M + K]$^+$, 397 [M + Na]$^+$, 375 [M + H]$^+$. Anal. ($C_{19}H_{23}N_2O_4P$) C, H, N. Calcd: C, 60.96; H, 6.19; N, 7.48. Found: C, 60.47; H, 6.48; N, 7.16.

(3*R*)-*N*-Hydroxy-2-[(*R*)-ethoxy(4-fluoroyphenyl)phosphoryl]-1,2,3,4-tetrahydroisoquinoline-3-carboxamide (34): colorless solids; 4% yield; ^{1}H NMR (DMSO-d_6) δ 1.00−1.40 (m, 3H), 2.90−3.15 (m, 2H), 3.70−3.90 (m, 1H), 3.90−4.10 (m, 1H), 4.10−4.40 (m, 2H), 4.40−4.55 (m, 1H), 7.00−7.20 (m, 4H), 7.25−7.45 (m, 2H), 7.80−7.95 (m, 2H), 8.80 (s, 1H), 10.62 (s, 1H); MALDI-TOF MS *m*/*z* 417 [M + K]$^+$, 401 [M + Na]$^+$, 379 [M + H]$^+$. Anal. ($C_{18}H_{20}FN_2O_4P$) C, H, N.

(3*R*)-*N*-Hydroxy-2-[(*R*)-ethoxy(3-fluorophenyl)phosphoryl]-1,2,3,4-tetrahydroisoquinoline-3-carboxamide (35): colorless solids; 18% yield; ^{1}H NMR (DMSO-d_6) δ 1.20 (t, J = 7.0 Hz, 3H), 2.90−3.10 (m, 2H), 3.70−3.90 (m, 1H), 3.90−4.10 (m, 1H), 4.19 (dd, J = 7.6 and 16.0 Hz, 1H), 4.31 (dd, J = 4.9 and 16.0 Hz, 1H), 4.45−4.55 (m, 1H), 7.05−7.20 (m, 4H), 7.40−7.50 (m, 1H), 7.50−7.70 (m, 3H), 8.84 (br s, 1H), 10.65 (br s, 1H); MALDI-TOF MS *m*/*z* 417 [M + K]$^+$, 401 [M + Na]$^+$, 379 [M + H]$^+$. Anal. ($C_{18}H_{20}FN_2O_4P$) C, H, N.

(3*R*)-*N*-Hydroxy-2-[(*R*)-ethoxy(2-fluorophenyl)phosphoryl]-1,2,3,4-tetrahydroisoquinoline-3-carboxamide (36): colorless solids; 8% yield; ^{1}H NMR (DMSO-d_6) δ 1.18 (t, J = 7.0 Hz, 3H), 2.91 (d, J = 4.6 Hz, 2H), 3.75−3.90 (m, 1H), 3.95−4.10 (m, 1H), 4.32 (dd, J = 8.1 and 16.3 Hz, 1H), 4.35−4.45 (m, 1H), 4.50 (dd, J = 4.3 and 16.3 Hz, 1H), 7.05−7.20 (m, 4H), 7.20−7.35 (m, 2H), 7.62 (q, J = 7.0 Hz, 1H), 7.75−7.95 (m, 1H), 8.78 (br s, 1H), 10.57 (br s, 1H); MALDI-TOF MS *m*/*z* 417 [M + K]$^+$, 401 [M + Na]$^+$, 379 [M + H]$^+$. Anal. ($C_{18}H_{20}FN_2O_4P$) C, H, N.

(3*R*)-*N*-Hydroxy-2-[(*R*)-ethoxy(4-pyridyl)phosphoryl]-1,2,3,4-tetrahydroisoquinoline-3-carboxamide (37): colorless solids; 13% yield; ^{1}H NMR (DMSO-d_6) δ 1.20 (t, J = 7.0 Hz, 3H), 2.85−3.20 (m, 2H), 3.75−3.95 (m, 1H), 3.95−4.10 (m, 1H), 4.15−4.35 (m, 2H), 4.40−4.55 (m, 1H), 7.05−7.25 (m, 4H), 7.70 (dd, J = 5.9 and 12.9 Hz, 2H), 8.70−8.80 (m, 2H), 8.82 (br s, 1H), 10.64 (br s, 1H); MALDI-TOF MS *m*/*z* 400 [M + K]$^+$, 384 [M + Na]$^+$, 362 [M + H]$^+$. Anal. ($C_{17}H_{20}N_3O_4P$) C, H, N.

(3*R*)-*N*-Hydroxy-2-[(*R*)-ethoxy(4-aminophenyl)phosphoryl]-1,2,3,4-tetrahydroisoquinoline-3-carboxamide trifluoroacetic acid salt (38): colorless solids; 12% yield; ^{1}H NMR (DMSO-d_6) δ 1.12 (t, J = 7.0 Hz, 3H), 2.85−2.95 (m, 2H), 3.60−3.80 (m, 1H), 3.80−4.00 (m, 1H), 4.13 (dd, J = 7.7 and 16.3 Hz, 1H), 4.25−4.45 (m, 2H), 5.70 (br s, 2H), 6.56 (dd, J = 3.5 and 8.5 Hz, 2H), 7.00−7.15 (m, 4H), 7.37 (dd, J = 8.5 and 12.5 Hz, 2H), 8.76 (s, 1H), 10.52 (s, 1H); MALDI-TOF MS *m*/*z* 414 [M + K]$^+$, 398 [M + Na]$^+$, 376 [M + H]$^+$. Anal. ($C_{20}H_{23}F_3N_3O_6P$) C, H, N.

(3*R*)-*N*-Hydroxy-2-[(*R*)-ethoxy(2-thienyl)phosphoryl]-1,2,3,4-tetrahydroisoquinoline-3-carboxamide (39): colorless solids; 6% yield; ^{1}H NMR (DMSO-d_6) δ 1.18 (t, J = 7.0 Hz, 3H), 2.70−3.20 (m, 2H), 3.75−4.00 (m, 2H), 4.25 (dd, J = 8.0 and 16.3 Hz, 1H), 4.30−4.50 (m, 2H), 7.00−7.30 (m, 5H), 7.65 (dd, J = 3.4 and 8.1 Hz, 1H), 7.96 (t, J = 4.8 Hz, 1H), 8.80 (s, 1H), 10.55(s, 1H); MALDI-TOF MS *m*/*z* 405 [M + K]$^+$, 389 [M + Na]$^+$, 367 [M + H]$^+$. Anal. ($C_{16}H_{19}N_2O_4PS$) C, H, N.

(3*R*)-*N*-Hydroxy-2-{(*R*)-ethoxy[4-(2-ethoxyethoxy)phenyl]phosphoryl}-1,2,3,4-tetrahydroisoquinoline-3-carboxamide (40): colorless solids; 8% yield; ^{1}H NMR (DMSO-d_6) δ 1.12 (t, J = 7.0 Hz, 3H), 1.15 (t, J = 7.1 Hz, 3H), 2.90−3.00 (m, 2H), 3.49 (q, J = 7.0 Hz, 2H), 3.65−3.85 (m, 3H), 3.85−4.05 (m, 1H), 4.05−4.25 (m, 3H), 4.25−4.50 (m, 2H), 6.95−7.15 (m, 6H), 7.69 (dd, J = 8.8 and 12.5 Hz, 2H), 8.78 (s, 1H), 10.57 (br s, 1H); MALDI-TOF MS *m*/*z* 487 [M + K]$^+$, 471 [M + Na]$^+$, 449 [M + H]$^+$. Anal. ($C_{22}H_{29}N_2O_6P$) C, H, N.

(3*R*)-*N*-Hydroxy-2-[(*R*)-ethoxy(4-biphenyl)phosphoryl]-1,2,3,4-tetrahydroisoquinoline-3-carboxamide (41): colorless solids; 16% yield; ^{1}H NMR (DMSO-d_6) δ 1.19 (t, J = 7.1 Hz, 3H), 2.85−3.15 (m, 2H), 3.70−3.90 (m, 1H), 3.90−4.10 (m, 1H), 4.22 (dd, J = 7.5 and 15.9 Hz, 1H), 4.37 (dd, J = 4.3 and 15.9 Hz, 1H), 4.45−4.55 (m, 1H), 7.00−7.20 (m, 4H), 7.30−7.60 (m, 3H), 7.60−7.95 (m, 6H), 8.81 (s, 1H), 10.62 (s, 1H); MALDI-TOF MS *m*/*z* 475 [M + K]$^+$, 459 [M + Na]$^+$, 437 [M + H]$^+$. Anal. ($C_{24}H_{25}N_2O_4P$) C, H, N.

(3*R*)-*N*-Hydroxy-2-{(*R*)-ethoxy[4-(pyridine-4-yl-oxy)-phenyl]phosphoryl}-1,2,3,4-tetrahydroisoquinoline-3-carboxamide trifluoroacetic acid salt (43): colorless solids; 9% yield; ^{1}H NMR (DMSO-d_6) δ 1.10−1.30 (m, 3H), 2.80−3.10 (m, 2H), 3.70−3.90 (m, 1H), 3.90−4.10 (m, 1H), 4.10−4.40 (m, 2H), 4.40−4.55 (m, 1H), 7.00−7.20 (m, 4H), 7.20−7.30 (m, 2H), 7.30−7.40 (m, 2H), 7.85−8.00 (m, 2H), 8.60−8.75 (m, 2H), 10.70 (s, 1H); MALDI-TOF MS *m*/*z* 492 [M + K]$^+$, 476 [M + Na]$^+$, 454 [M + H]$^+$. Anal. ($C_{25}H_{25}F_3N_3O_7P$) C, H, N.

(3*R*)-*N*-Hydroxy-2-{(*R*)-ethoxy[4-(4-aminophenyloxy)-phenyl]phosphoryl}-1,2,3,4-tetrahydroisoquinoline-3-carboxamide (44): colorless solids; 18% yield; ^{1}H NMR (DMSO-d_6) δ 1.15 (t, J = 7.1 Hz, 3H), 2.85−3.05 (m, 2H), 3.65−3.80 (m,1H), 3.85−4.00 (m, 1H), 4.05−4.20 (m, 1H), 4.25−4.35 (m, 1H), 4.35−4.50 (m,1H), 6.95−7.15 (m, 10H), 7.74 (d, J = 12.5 Hz, 1H), 7.76 (d, J = 12.5 Hz, 1H), 10.59 (s,1H); MALDI-TOF MS *m*/*z* 506 [M + K]$^+$, 490 [M + Na]$^+$, 468 [M + H]$^+$. Anal. ($C_{24}H_{26}N_3O_5P$) C, H, N.

(3*R*)-*N*-Hydroxy-2-[(*R*)-ethoxy(styryl)phosphoryl]-1,2,3,4-tetrahydroisoquinoline-3-carboxamide (45): colorless solids; 6% yield; ^{1}H NMR (DMSO-d_6) δ 1.15 (t, J = 7.1 Hz, 3H), 3.00−3.10 (m, 2H), 3.70−3.85 (m, 1H), 3.85−4.05 (m, 1H), 4.23 (dd, J = 7.1 and 16.2 Hz, 1H), 4.30−4.50 (m, 2H), 6.62 (t, J = 17.9 Hz, 1H), 7.05−7.20 (m, 4H), 7.20−7.50 (m, 4H), 7.55−7.65 (m, 2H), 8.78 (s, 1H), 10.56 (br s, 1H); MALDI-TOF MS m/z 425 [M + K]$^+$, 409 [M + Na]$^+$, 387 [M + H]$^+$. Anal. ($C_{20}H_{23}N_2O_4P$) C, H, N.

(3*R*)-*N*-Hydroxy-2-[(*R*)-ethoxy(2-phenylethyl)phosphoryl]-1,2,3,4-tetrahydroisoquinoline-3-carboxamide (46): colorless solids; 29% yield; ^{1}H NMR (DMSO-d_6) δ 1.08 (t, J = 7.0 Hz, 3H), 1.95−2.25 (m, 2H), 2.60−2.90 (m, 2H), 2.95−3.15 (m, 2H), 3.50−3.75 (m, 1H), 3.75−3.95 (m, 1H), 4.21 (dd, J = 6.8 and 16.0 Hz, 1H), 4.30−4.50 (m, 2H), 7.05−7.35 (m, 9H), 8.77 (s, 1H), 10.56 (s, 1H); MALDI-TOF MS m/z 427 [M + K]$^+$, 411 [M + Na]$^+$, 389 [M + H]$^+$. Anal. ($C_{20}H_{25}N_2O_4P$) C, H, N.

General Procedure for Preparing Phosphonamide Derivatives (Method D). Synthesis of (3*R*)-*N*-Hydroxy-2-[(*R*)-ethoxy(4-methoxyphenyl)phosphoryl]-7-amino-1,2,3,4-tetrahydroisoquinoline-3-carboxamide (47). Diastereomers of (3*R*)-*N*-Benzyloxy-2-[(*R*)-ethoxy(4-methoxyphenyl)phosphoryl]-7-nitro-1,2,3,4-tetrahydroisoquinoline-3-carboxamide (16a). To a solution of (4-methoxyphenyl)chlorophosphonic acid ethyl ester **15** (470 mg, 2.00 mmol, prepared from (4-methoxyphenyl)phosphonic acid diethyl ester) in 7 mL of dry CH_2Cl_2 was added (3*R*)-*N*-benzyloxy-7-nitro-1,2,3,4-tetrahydroisoquinole-3-carboxamide **14a** (628 mg, 1.92 mmol) and diisopropylethylamine (1 mL), and the mixture was stirred at room temperature under an argon atmosphere for 14 h. The reaction mixture was diluted with $CHCl_3$. The solution was washed successively with water, saturated $NaHCO_3$, and brine and dried over $MgSO_4$. The solvent was evaporated, and the residue was purified by column chromatography on silica gel, eluting with $CHCl_3$/MeOH 50:1 to give the title compound **16a** as a diasteromeric mixture (1:1, 820 mg, 81%): ^{1}H NMR ($CDCl_3$) δ 1.23 (t, J = 7.0 Hz, 1.5H), 1.34 (t, J = 7.0 Hz, 1.5H), 2.75−3.05 (m, 1H), 3.35−3.55 (m, 1H), 3.80−3.90 (m, 4H), 3.90−4.20 (m, 3H), 4.20−4.40 (m, 2H), 4.55−4.65 (m, 0.5H), 4.80−4.95 (m, 2H), 6.85−7.05 (m, 2H), 7.36 (br, 5H), 7.45−7.90 (m, 4H), 7.95−8.10 (m, 1H), 9.99 (br, 0.5H), 10.31 (br, 0.5H).

(3*R*)-*N*-Hydroxy-2-[(*R*)-ethoxy(4-methoxyphenyl)phosphoryl]-7-amino-1,2,3,4-tetrahydroisoquinoline-3-carboxamide (47). The title compound was prepared from **16a** following the precedure described for compound **19**: yield 16%; ^{1}H NMR (DMSO-d_6) δ 1.16 (qn, J = 6.5 Hz, 3H), 2.75−3.10 (m, 2H), 3.70−3.90 (m, 3H), 3.90−4.10 (m, 2H), 4.10−4.35 (m, 2H), 4.35−4.50 (m, 1H), 6.45−6.75 (m, 1H), 6.79 (s, 1H), 6.85 (d, J = 8.3 Hz, 1H), 6.90−7.10 (m, 2H), 7.50−7.75 (m, 2H), 8.70 (br, 1H), 10.59 (br, 1H); MALDI-TOF MS m/z 444 [M + K]$^+$, 428 [M + Na]$^+$, 406 [M + H]$^+$. Anal. ($C_{19}H_{24}N_3O_5P$) C, H, N.

Compounds **48** and **52** were also synthesized using a procedure similar to the procedure for the preparation of **47**. The corresponding amines **14a** were prepared from the corresponding amino acids utilizing the Pictet−Spengler reaction condition.[21]

***N*-Hydroxy-6-[(*R*)-ethoxy(4-methoxyphenyl)phosphoryl]-5,6,7,8-tetrahydropyrido[3,4-*b*]pyrazine-7-carboxamide (48).** The title compound was prepared from (+)-*N*-benzyloxy-5,6,7,8-tetrahydropyrido[3,4-*b*]pyrazine-7-carboxamide hydrochloride[24] following the precedure described for compound **19**: yield 16%; colorless solids; 20% yield; ^{1}H NMR (DMSO-d_6) δ 1.20 (t, J = 7.0 Hz, 3H), 2.90−3.25 (m, 2H), 3.84 (s, 3H), 3.75−4.15 (m, 2H), 4.25−4.50 (m, 2H), 4.50−4.65 (m, 1H), 7.00−7.10 (m, 2H), 7.60−7.70 (m, 2H), 8.40−8.45 (m, 2H), 8.90 (br s, 1H), 10.84 (br s, 1H); MALDI-TOF MS m/z 431 [M + K]$^+$, 415 [M + Na]$^+$, 393 [M + H]$^+$. Anal. ($C_{17}H_{21}N_4O_5P$) C, H, N.

(2*R*)-*N*-Hydroxy-1-[(*R*)-ethoxy(4-methoxyphenyl)phosphoryl]pyrrolidine-2-carboxamide (52): colorless solids; 7% yield; ^{1}H NMR (DMSO-d_6) δ 1.27 (t, J = 7.1 Hz, 3H), 1.60−2.00 (m, 4H), 3.00−3.20 (m, 2H), 3.80 (s, 3H), 3.85−4.15 (m, 3H), 7.02 (dd, J = 3.0 and 8.7 Hz, 2H), 7.68 (dd, J = 8.7, 12.2 Hz, 2H), 8.79 (br s, 1H), 10.43 (br s, 1H); MALDI-TOF MS m/z 367 [M + K]$^+$, 351 [M + Na]$^+$, 329 [M + H]$^+$. Anal. ($C_{14}H_{21}N_2O_5P$) C, H, N.

Compounds **16b** were synthesized from the corresponding methyl ester derivatives **14b** instead of from benzyloxyamide derivatives **14a** using a procedure similar to the procedure for the preparation of **16a**. The methyl esters **16b** required the following base hydrolysis and amidation to obtain the corresponding hydroxamic acids **49** and **51**.

(3*R*)-*N*-Hydroxy-5-[(*R*)-ethoxy(4-methoxyphenyl)phosphoryl]-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine-6-carboxamide (49). To a solution of 5-[ethoxy-(4-methoxyphenyl)-phosphinoyl]-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine-6-carboxylic acid methyl ester **16b** (0.60 g, 1.5 mmol) in 6 mL of DME was added 3 mL of 1 N NaOH, and the solution was stirred for 3 h. The reaction mixture was then neutralized with 1 N HCl and extracted with EtOAc. The organic layer was washed with water, dried over Na_2SO_4, and evaporated to afford 0.49 g of the corresponding carboxylic acid. To a solution of the resulting acid (0.49 g, 1.3 mmol) and triethylamine (1.0 g, 9.9 mmol) in DMF (10 mL) was added WSC (0.31 g, 1.6 mmol) and HOBt (0.25 g, 1.6 mmol) at 0 °C, and the mixture was stirred for 30 min. Then, hydroxylamine hydrochloride (1.0 g, 14 mmol) was added to the reaction mixture, and the stirring was continued overnight. The reaction mixture was diluted with water and extracted with AcOEt. The organic layer was dried over Na_2SO_4 and evaporated. The residue was purified by HPLC (YMC-ODS, CH_3CN/water 72:28), and the fraction eluted first was lyophilized to afford the title compound **49** (53 mg, 10%) as coloress solids: ^{1}H NMR (DMSO-d_6) δ 1.18 (t, J = 7.0 Hz, 3H), 2.90−3.15 (m, 2H), 3.82 (s, 3H), 3.70−4.45 (m, 4H), 4.62 (dd, J = 5.6 and 9.7 Hz, 1H), 6.81 (d, J = 5.2 Hz, 1H), 7.04 (dd, J = 3.1 and 8.7 Hz, 2H), 7.27 (d, J = 5.2 Hz, 1H), 7.72 (dd, J = 8.7 and 12.5 Hz, 2H), 8.80 (s, 1H), 10.58 (s, 1H); MALDI-TOF MS m/z 435 [M + K]$^+$, 419 [M + Na]$^+$, 397 [M + H]$^+$. Anal. ($C_{17}H_{21}N_2O_5PS$) C, H, N.

(2*R*)-*N*-Hydroxy-4-benzyloxycarbonyl-1-[ethoxy(4-methoxyphenyl)phosphoryl]piperazine-2-carboxamide (51): colorless solids; 8% yield; ^{1}H NMR (DMSO-d_6) δ 1.25 (t, J = 7.0 Hz, 3H), 2.85 (br, 1H), 3.00−3.15 (m, 2H), 3.30−3.50 (m, 1H), 3.79 (s, 3H), 3.86 (d, J = 11.8 Hz, 1H), 3.97 (q, J = 10.0 Hz, 2H), 4.05−4.15 (m, 1H), 4.18 (d, J = 13.6 Hz, 1H), 5.03 (br, 2H), 7.01 (dd, J = 3.1 and 8.8 Hz, 2H), 7.20−7.40 (m, 5H), 7.69 (dd, J = 8.8 and 12.4 Hz, 2H), 8.86 (br, 1H), 10.57 (br, 1H); MALDI-TOF MS m/z 516 [M + K]$^+$, 500 [M + Na]$^+$, 478 [M + H]$^+$. Anal. ($C_{22}H_{28}N_3O_7P$) C, H, N.

2. Metalloproteinase Inhibition Assay. 2.1. MMP Inhibition Assay. DNA fragments coding the catalytic domain of human MMP-1 and human MMP-9 and a DNA fragment coding from the prodomain to the catalytic domain of human MMP-3 were amplified by polymerase chain reaction (PCR) from cDNA of HT1080 cells stimulated with 0.01 μM of TPA. The 5′-end of each PCR primer was added to a sequence for the appropriate restriction enzyme site. Amplified DNA fragments were cloned into a cloning vector and then introduced into a commercially available expression vector containing a His-6 tag sequence at the end of the N-terminus. Recombinant proteins were expressed in *E. coli* cells and purified by Ni−NTA resin (Qiagen Inc.) and refolded. Recombinant MMP-3 was activated by incubation with 1 mM *p*-aminophenylmercuric acetate for 1 h at 37 °C. Test compounds were dissolved in DMSO and diluted with reaction buffer (50 mM Tris, 150 mM NaCl, 10 mM $CaCl_2$, 0.05% Brij-35, pH 7.5). A total of 25 μL of compound solution was mixed with 25 μL of diluted enzyme solution in a well of a 96-well half-area black microplate (COSTAR) and was incubated for 10 min at 37 °C. The reaction was started by adding 50 μL of fluorescence-quenching peptide substrate solution to the well, and incubated for 2 (MMP-1 and MMP-3) or 3 (MMP-9) h at 37 °C. A total of 5 μM of MOCAc-Pro-Leu-Gly-Leu-A2pr(Dnp)-Ala-Arg-NH_2[32] (Peptide Institute, Inc.) was used as a substrate for MMP-1 and MMP-9, and 5 μM of MOCAc-Arg-Pro-Lys-Pro-Val-Glu-Nva-Trp-Arg-Lys(Dnp)-NH_2[33] (Peptide Institute, Inc.) was used as

a substrate for MMP-3. After incubation, fluorescence intensities (Ex/Em = 320/405 nm) of the wells were measured by a fluorescence microplate reader (Polarstar; BMG LabTechnologies, Germany). K_i values were calculated from the percent inhibition and the K_m value of each MMP to the substrate by using GraphPad Prism.

2.2 TACE Inhibition Assay. The DNA fragment coding signal region, prodomain and catalytic domain of TACE, was amplified by polymerase chain reaction from pBluescriptII-TACE clone (cloned a full-length cDNA for TACE from human acute monocytic leukemia cell line, THP-1) as the template. The 5′-end of each PCR primer was added to a sequence for the appropriate restriction enzyme site, and the FLAG tag sequence was also added to the reverse primer. The amplified DNA fragment was cloned into pFastBac-1 transfer vector (Life Technologies, Rockville, MD). pFastBac-1/TACE was transformed into DH10Bac cells (Life Technologies, Rockville, MD), and the recombinant bacmid was isolated, purified, and then used to generate baculovirus particles in Sf9 insect cells (Pharmingen, San Diego, CA). Logarithmically growing Sf9 cells were infected with TACE baculovirus at a MOI1. Conditioned media were harvested at 96 h after infection. The recombinant TACE (rTACE) was purified from the medium with an anti-FLAG M2 affinity gel column (Sigma, St. Louis, MO). Purified rTACE had an approximately 90% purity.

The rTACE (final 50 ng/mL) was mixed with the compound solution and incubated at 37 °C for 10 min in a reaction buffer (20 mM Tris-HCl (pH 7.5) containing 0.05% Brij-35). The reaction was initiated by addition of 5 μM (final concentration) of fluorescence-quenching peptide substrate (Mca-Pro-Leu-Ala-Glu-Ala-Val-Dap(Dnp)-Arg-Ser-Ser-Ser-Arg-NH_2; Bachem AG, Switerland),[34] which contained the cleavage site of proTNF-α, and the increase of fluorescence intensity (Ex/Em = 320/405 nm) was monitored. K_i values were calculated from the percent inhibition and the K_m value of rTACE to the substrate by using GraphPad Prism, version 3.0 (GraphPad Software, Inc., San Diego, CA). The K_m value of purified rTACE was about 17 μM, and this value was very similar to that of the previous report.[35]

2.3. HB-EGF Shedding Assay.[25] The expression vector of HB-EGF fused with human placental alkaline phosphatase (AP) that was constructed as described previously[25] was obtained from Dr. Higashiyama (School of Medicine, Osaka University, Osaka, Japan). Fibrosarcoma HT-1080 transfectants expressing AP-tagged HB-EGF in MEM (containing 10% FCS) as the culture medium was seeded in 96-well plates at a density of 2×10^5 cells/well and incubated for 24 h. The cells were washed with PBS and preincubated with test compounds in MEM (containing 1% DMSO) for 30 min. TPA (60 nM) was added to stimulate inducible processing, and the plate was incubated for 60 min. A 0.1 mL aliquot of the supernatant was transferred to 96-well plates and heated for 10 min at 65 °C in order to inactivate endogenous alkaline phosphatases. A 0.1 mL of substrate solution (1 M diethanolamine, 0.01% $MgCl_2$, 1 mg/mL *p*-nitrophenyl phosphate, pH 9.8) was added to each well, and the plates were incubated for 2 h. The AP activity was then determined by the measurement of absorbance at 405 nm with a microplate reader. The IC_{50} value was determined with different inhibitor concentrations by using GraphPad Prism, version 3.0 (GraphPad Software, Inc.).

3. Molecular Modeling. A model of the MMP-3 catalytic domain (CD) complexed with phosphonamide inhibitor was constructed on the basis of the crystal structure of a MMP-3−phosphinamide complex (PDB code 1B3D). Modeling work was performed using the program SYBYL. The phosphinamide inhibitor **1** in the complex was replaced by the phosphonamide inhibitor **19** by superimposing hydroxamate and the phosphorus atom of the phosphonamide inhibitor on those of the phosphinamide inhibitor. Then, the protein−inhibitor complex was minimized by treating all ligand atoms.

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