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Synthesis and biological activity of sulphostin analogues, novel dipeptidyl peptidase IV inhibitors

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Abstract—The structure of sulphostin (**1**), a novel dipeptidyl peptidase IV (DPP-IV) inhibitor, is consisted of three key functional groups, including a characteristic amino(sulfoamino)phosphinyl group, on a piperidine ring. To examine the relationship between its structure and the inhibitory activity against DPP-IV, various analogues were synthesized and their activities were investigated. These results indicated that all of the functional groups on the piperidine ring were crucial to the DPP-IV inhibitory activity of sulphostin, and that the sulfonic acid group, which constructed the amino(sulfoamino)phosphinyl group, contributed to the stability of the compound. Moreover, those functional groups should be adjoined on the piperidine ring for the inhibitory activity. The size of the nitrogen-containing heterocyclic ring including piperidine appeared to scarcely affect the activity. In the present study, the sulfonic acid-deficient five-membered ring analogue **27a** showed the strongest inhibitory activity (IC_{50} = 11 nM).

1. Introduction

Dipeptidyl peptidase IV (DPP-IV, CD26, EC 3.4.14.5) is a cell-surface serine exopeptidase that selectively cleaves dipeptide from polypeptides, including proline or alanine residues at the N-terminal penultimate position.[1] This enzyme cleaves several important polypeptides such as chemokines and peptide hormones.[2] Glucagon-like peptide-1 (7–36 amide, GLP-1), an insulin-releasing hormone, is also cleaved and inactivated by DPP-IV.[3] Based on an examination of the relationship of DPP-IV with insulin in vivo, it has been confirmed that DPP-IV inhibitors suppress the rise in glucose level in blood after a meal.[4] NVP-DPP728[5] and P32/98[6] (Fig. 1), which are potent DPP-IV inhibitors, are currently being evaluated in clinical trials as therapeutic agents against type 2 diabetes. In the immune system, CD26, which is identical to DPP-IV, has been reported to affect the proliferation and activation of T cells.[7] It has been confirmed that two DPP-IV inhibitors, prodipine[8] and Ala-boroPro[9] (Fig. 1), suppress the immune response in vivo. Therefore, the DPP-IV inhibitor has been expected to be an effective therapeutic agent for type 2 diabetes and/or immune-related disorders. A wide range of compounds have shown the inhibitory activity of DPP-IV. The structural features of the most potent inhibitors, however, including the four above-mentioned synthetic inhibitors, have Xaa-Pro (Xaa: any amino acid) mimic. As for one of natural products with the DPP-IV inhibitory activity, diprotins (A: Ile-Pro-Ile, B: Val-Pro-Leu),

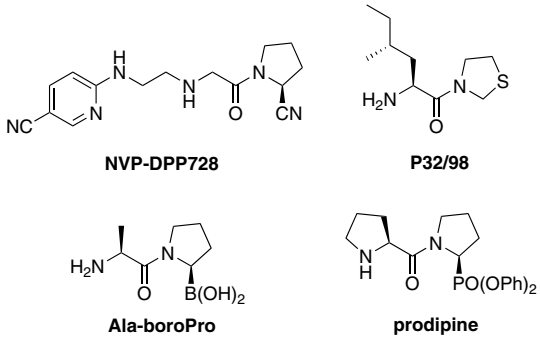

Figure 1. Structures of DPP-IV inhibitors of Xaa-Pro derivative.

Keywords: Sulphostin analogues; Dipeptidyl peptidase IV inhibitors; Structure–activity relationships.
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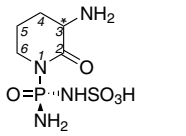


*S : sulphostin (1)
*R : 3-epi sulphostin (2)

Figure 2. Structures of sulphostin (**1**) and 3-*epi* sulphostin (**2**).

including a proline residue at the N-terminal penultimate position, were reported by Umezawa and co-workers in our institute.[10]

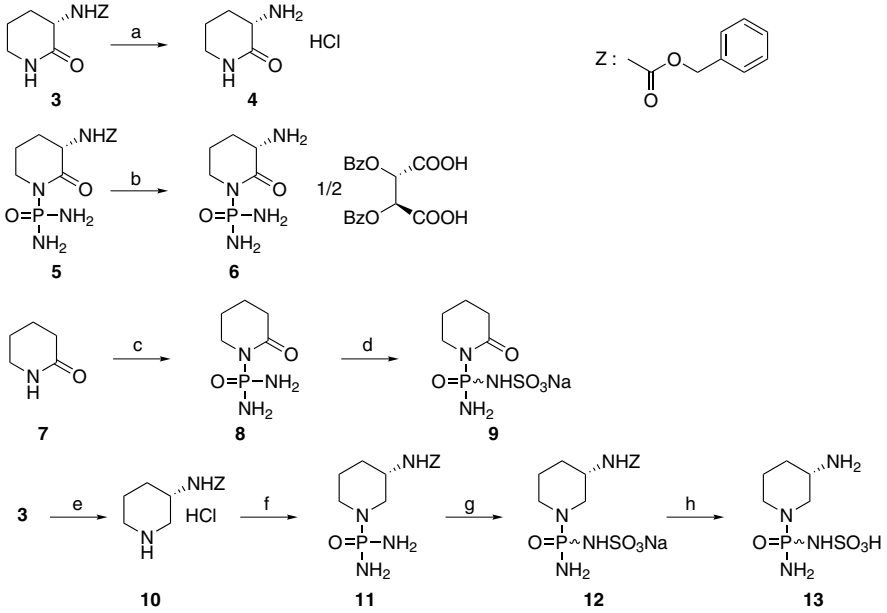
We recently isolated sulphostin (**1**), which had a DPP-IV inhibitory activity, from the culture broth of *Streptomyces* sp. MK251-43F3 (Fig. 2).[11] Sulphostin contains two asymmetric atoms at C-3 and phosphorus in its structure. The absolute configuration of the natural product was determined by a comparison with the four synthesized stereoisomers.[12] Among four stereoisomers, sulphostin and 3-epi sulphostin (**2**), in which the configuration at the phosphorus atom was *R*, showed strong DPP-IV inhibitory activities. The basic skeleton of sulphostin is entirely different from those of the other reported DPP-IV inhibitors containing Xaa-Pro derivatives. We therefore decided to examine the relationship between the structure of sulphostin and the DPP-IV inhibitory activity. The various analogues of sulphostin, namely the functional group-deficient analogues, the functional group position-changed analogues, and the ring size-changed analogues, were synthesized, and their inhibitory activities against DPP-IV were measured. We report herein our detailed findings regarding the structure–activity relationships of sulphostin and its analogues.

2. Chemistry

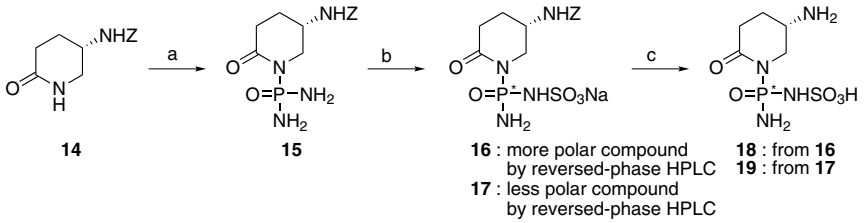
Synthetic methods of the functional group-deficient analogues of sulphostin are shown in Scheme 1. The amino(sulfoamino)phosphinyl group-deficient analogue **4** and the sulfonic acid group-deficient analogue **6** were prepared by catalytic hydrogenolysis with palladium-black from (3*S*)-3-(Z-amino)-2-piperidinone (Z: benzyloxycarbonyl) (**3**) and (3*S*)-3-(Z-amino)-1-diaminophosphinyl-2-piperidinone (**5**), respectively, which were synthetic intermediates of sulphostin.[12]

The 3-amino group-deficient analogue **9** was synthesized as a racemic sodium salt from 2-piperidinone (**7**) in two steps; as follows. The lactam amide of 2-piperidinone (**7**) was activated by *n*-butyllithium and was treated with phosphoryl chloride. Subsequent reaction of the dichlorophosphinyl intermediate with liquid ammonia gave 1-diaminophosphinyl-2-piperidinone (**8**). After sulfonation with pyridine/sulfur trioxide complex, the resultant racemic pyridinium salt was changed into the sodium salt by Dowex® 50W × 8 (Na form) to give the target compound **9**.

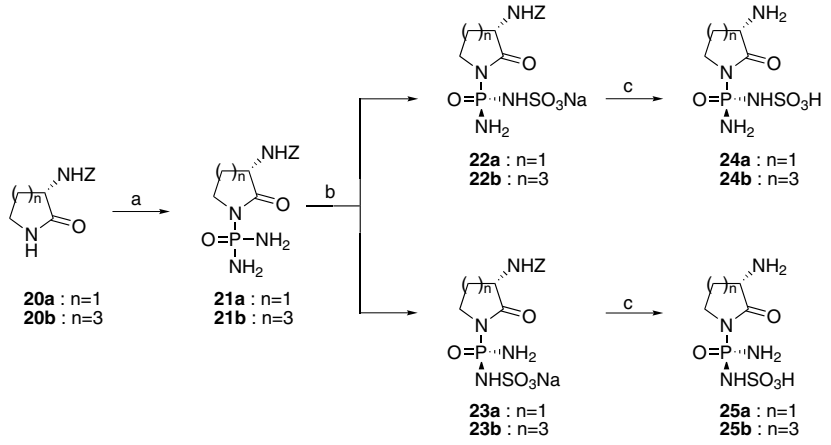
The 2-carbonyl group-deficient analogue **13** was synthesized as a diastereomeric mixture from (3*S*)-3-(Z-amino)piperidine hydrochloride (**10**), which was prepared by reduction of compound **3** using diisobutylaluminum hydride, in four steps. Compound **10** was treated with triethylamine and phosphoryl chloride, followed by liquid ammonia to give compound **11**. After sulfonation of compound **11**, the treatment with sodium hydrogen carbonate gave the sulfonic acid sodium salt (**12**) as a diastereomeric mixture. The diastereomeric mixture was purified using Diaion® HP-20SS column chromatography, and resultant sodium salt was changed



Scheme 1. Reagents and conditions: (a) (i) H_2/Pd-black, 20 h, (ii) HCl, 93%; (b) (i) H_2/Pd-black, 6 h, (ii) (+)-dibenzoyl-D-tartaric acid, 87%; (c) (i) *n*-BuLi, (ii) $POCl_3$, (iii) NH_3, −78 °C, 33%; (d) (i) SO_3·Py, 2 h, 6–8 °C, (ii) $NaHCO_3$, 41%; (e) (i) $^iBu_2AlH$, 4 h, (ii) HCl, 36%; (f) (i) Et_3N, $POCl_3$, (ii) NH_3, 0 °C, 57%; (g) SO_3·Py, 5 h, 6–8 °C, (ii) $NaHCO_3$, 47%; (h) (i) $BnNH_2$·HCl, (ii) H_2/Pd-black, 15 h, 79%.



Scheme 2. Reagents and conditions: (a) (i) *n*-BuLi, (ii) $POCl_3$, (iii) NH_3, −78 °C, 61%; (b) (i) SO_3·Py, 4 h, 6–8 °C, (ii) $NaHCO_3$, (iii) separation, **16**: 18%, **17**: 16%; (c) (i) $BnNH_2$·HCl, (ii) H_2/Pd-black, 4 h, **18**: 58%, **19**: 63%.

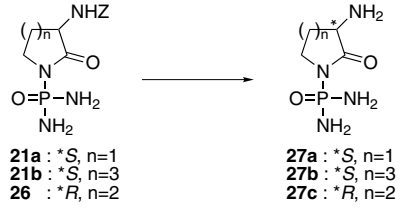


Scheme 3. Reagents and conditions: (a) (i) *n*-BuLi, (ii) $POCl_3$, (iii) NH_3, −78 °C, **21a**: 46%, **21b**: 30%; (b) (i) SO_3·Py, 4 h, 6–8 °C, (ii) $NaHCO_3$, (iii) separation, **22a**: 18%, **23a**: 20% from **21a**, **22b**: 18%, **23b**: 20% from **21b**; (c) (i) $BnNH_2$·HCl, (ii) H_2/Pd-black, 4 h, **24a**: 20%, **25a**: 25%, **24b**: 38%, **25b**: 42%.

into benzylamine salt for ease of desalination at final purification. Following hydrogenolysis and desalination gave the target compound **13**.

The synthetic method leading to the functional group position-changed analogues of sulphostin is shown in Scheme 2. (5*S*)-5-(Z-amino)-2-piperidinone (**14**), which was prepared from Z-L-glutamic acid γ-methyl ester by the reported method,[13] was selected as a starting material. The target compounds **18** and **19** were synthesized from compound **14** in four steps: diaminophosphinylation, sulfonation, separation of the diastereomeric mixture using Diaion® HP-20SS column chromatography, and deprotection. Though each diastereomer was obtained in pure form, determination of the stereochemistry was not performed.

The synthetic method for obtaining the ring size-changed analogues of sulphostin is shown in Scheme 3. For a starting material leading to the five-membered ring analogues **24a** and **25a**, (3*S*)-3-(Z-amino)-2-pyrrolidinone (**20a**), which was prepared from Z-L-glutamine by the method previously reported,[14] was selected. For the seven-membered ring analogues **24b** and **25b**, (3*S*)-3-(Z-amino)-2-perhydroazepinone (**20b**), which was easily prepared by protection of commercially available (3*S*)-3-amino-2-perhydroazepinone, was selected. The target compounds **24a**, **24b**, **25a**, and **25b** were synthesized from compounds **20a** and **20b** by a method similar to that noted for compounds **18** and **19**.



Scheme 4. Reagents and conditions: **27a**: H_2/Pd-black, 5 h, 54%; **27b**: (i) H_2/Pd-black, (ii) (+)-dibenzoyl-D-tartaric acid, 85%; **27c**: (i) H_2/Pd-black, (ii) (−)-dibenzoyl-L-tartaric acid, 83%.

The synthetic method leading to the sulfonic acid group-deficient five- and seven-membered ring analogues and the 3-*epi* sulphostin analogue is shown in Scheme 4. The target compounds **27a** and **27b** were able to prepare both as a free amine and as a 1/2 (+)-dibenzoyl-D-tartaric acid salt from compounds **21a** and **21b**, respectively. The target compound **27c** with an *R* configuration at C-3 was prepared as a 1/2 (−)-dibenzoyl-L-tartaric acid salt from compound **26**.[12]

3. In vitro assay for DPP-IV

DPP-IV was prepared from rat kidney homogenate by ammonium sulfate precipitation (30–80%).[15] The inhibitory activities of the analogues were determined by

minor modification of the reported method.[15] Gly-Pro-β-naphthylamide was used as a substrate, and the absorbance at 525 nm of the chromophore complex of β-naphthylamine, which was formed by hydrolysis of the substrate, was measured.

4. Results and discussion

As shown in Figure 2, the basic skeleton of the sulphostin structure is a piperidine ring with three functional groups: the characteristic amino(sulfoamino)phosphinyl group at N-1, the carbonyl group at C-2, and the amino group at C-3. As the role of each functional group on the ring against the DPP-IV inhibitory activity has not been known, the effects of these functional groups on sulphostin were explored by synthesizing sulphostin analogues. The DPP-IV inhibitory activity of the functional group-deficient analogues, in which the functional group was replaced with a hydrogen atom, was measured to confirm the interaction with DPP-IV and the necessity of the functional group (Table 1). The amino(sulfoamino)phosphinyl group-deficient analogue **4** showed no inhibitory activity completely. NVP-DPP728, Ala-boroPro, and prodipine have a nitrile group, a boronic acid group, and a diphenoxyphosphoryl group at C-2 on the pyrrolidine ring, respectively (in Fig. 1), as a functional group for making the coordinate bond with the hydroxyl group of ^{630}Ser, which is the active center of DPP-IV. Sulphostin does not have the above-mentioned functional groups; it does, however, have the amino(sulfoamino)phosphinyl group containing a phosphorus atom, which acquires a strong affinity for an oxygen atom. Therefore, it was presumed that the phosphorus atom in the amino(sulfoamino)phosphinyl group interacted with ^{630}Ser of the enzyme.

The amino(sulfoamino)phosphinyl group is constructed by the diaminophosphinyl group and the sulfonic acid group, and the role of the sulfonic acid group was not clear. Therefore, the sulfonic acid-deficient analogue was investigated to confirm the necessity of this functional group. The inhibitory activity of analogue **6** was maintained, and its IC_{50} value was 94 nM, which was approximately one-fourth of that of the inhibitory activity of parent sulphostin. Though, synthetic sulphostin was stable in solution, analogue **6** was appreciably unstable in both acidic and basic media. It seemed that the sulfonic acid group contributed not to the inhibitory activity but to the stability of the compound. The phosphorus atom of analogue **6** became asymmetric by the introduction of the sulfonic acid group, and one of the stereoisomers lost its DPP-IV inhibitory activity.[12]

Table 1. IC_{50} values of functional group-deficient analogues against DPP-IV

Compounds	IC_{50} (nM)
4	>100,000
6	94
9	>100,000
13	>100,000
1	21

From the X-ray crystal analyses of sulphostin (**1**) and P-*epi* sulphostin, oxygen atom of the phosphinyl group was away from the oxygen atom of the carbonyl group at C-2.[12] This positional relationship might attribute to the electric repulsion between two oxygen atoms, and might be retained even in solution. Therefore, it was considered that the configuration of the phosphorus atom was important to interact with the active site of DPP-IV because the conformation of the amino(sulfoamino)phosphinyl group was restricted by the carbonyl group at C-2. In the case of NVP-DPP728,[16] Val-boro-Pro,[17] and prodipine,[18] the compounds with an *R* configuration at C-2 on the piperidine ring showed stronger inhibitory activity than corresponding *S*-epimers. DPP-IV recognizes the configuration at the N-terminal penultimate position in the substrates and selectively cleaves only the L-amino acid residues.[1] The crucial asymmetric atom of sulphostin for the inhibitory activity is phosphorus, and its configuration must be *R* for keeping the conformation similar to that of the natural substrates.

The amino group-deficient analogue **9** showed no inhibitory activity completely. In addition, the *N*-Z-sulphostin analogue, in which the amino group at C-3 was protected as a urethane, similarly lost its DPP-IV inhibitory activity. These results indicate that the amino group at C-3 is quite important to the interaction with DPP-IV as well as the reported dipeptide inhibitors.[19] However, the configuration at C-3 did not appreciably affect the enzyme inhibitory activity.[12] It was thought that the conformation of the piperidine ring changed so that the amino group turned to the direction of pseudo equatorial.

In the case of the carbonyl group-deficient analogue, analogue **13** also showed no inhibitory activity completely. This carbonyl group at C-2 was similarly necessary together with the amino group at C-3, as a dipeptide mimic. It was confirmed that all of the functional groups on the piperidine ring of sulphostin are important to exhibit its inhibitory activity.

We next examined the positional arrangement of the functional groups. The carbonyl group should be placed adjacent to the amino(sulfoamino)phosphinyl group at N-1 to maintain the conformation of the phosphorus atom for its interaction with DPP-IV. We therefore selected 5-aminosulphostin analogues **18** and **19**, which the amino group at C-3 moved to C-5 keeping the distance from the phosphorus atom, for the examination. Both analogues **18** and **19** completely showed no DPP-IV inhibitory activities (IC_{50} = >100,000 nM). Consequently, it was confirmed that the amino(sulfoamino)phosphinyl group, the carbonyl group, and the amino group should adjoin on the piperidine ring to exhibit the DPP-IV inhibitory activity.

Many studies concerning DPP-IV inhibitors have been carried out using dipeptide derivatives such as Xaa-Pro. Among these studies, Augustyns et al. examined the size of the heterocyclic ring based on L-isoleucylpyrrolidine and reported that the inhibitory activity of the

Table 2. IC_{50} values of ring size-changed analogues against DPP-IV

Compounds	*n*	Config. at P	IC_{50} (nM)
24a	1	*R*	14
25a	1	*S*	1700
24b	3	*R*	25
25b	3	*S*	2200
1	2	*R*	21
P-epimer of 1	2	*S*	100,000

dipeptide derivative diminished with increases in the size of the heterocyclic ring.[20] Because the basic skeleton of sulphostin is a six-membered ring, were interested in the ring size-changed analogues. The DPP-IV inhibitory activities of the five- and seven-membered ring analogues are shown in Table 2. Both the five-membered ring analogue **24a** and the seven-membered ring analogue **24b** exhibited strong inhibitory activities, with IC_{50} values of 14 and 25 nM, respectively. Sulphostin, in which the configuration of the phosphorus atom is *R*, possesses a strong inhibitory activity; however, P-*epi* sulphostin, in which the configuration of the phosphorus atom is *S*, exhibits only marginal inhibitory activity.[12] From the biological activities, it was presumed that the configuration of the phosphorus atom in analogues **24a** and **24b**, which showed strong activities, was *R*, while that of analogues **25a** and **25b**, which showed weak activities, was *S*. These results indicated that the effect of the difference in the ring size with regard to the DPP-IV inhibitory activity was negligible within the limits of five-, six-, and seven-membered ring compounds.

Because both the ring size-changed analogues and the sulfonic acid group-deficient analogue maintained their inhibitory activities, the sulfonic acid group-deficient analogues of five- and seven-membered ring and 3-*epi* sulphostin (**2**), for which the IC_{50} value was 31 nM,[12] were investigated (Table 3). The inhibitory activities of the seven-membered ring analogue **27b** and the 3-*epi* analogue **27c** were slightly weak in comparison with parent compounds **24b** and **2**, and these results were similar to that of the six-membered ring analogue. However, the five-membered ring analogue **27a** showed the stronger inhibitory activity than the parent five-membered ring analogue **24a**. It had an IC_{50} value of 11 nM, which was the strongest activity in the present study. Among the sulfonic acid group-deficient analogues, it seemed that the five-membered ring analogue was the most desirable with regard to the positional relationship of the functional groups on the ring. The stabilities of analogues **27a**–**c** in solution were worse similar to that of analogue **6** than that of their parent compounds.

Table 3. IC_{50} values of sulfonic acid-deficient analogues against DPP-IV

Compounds	*n*	Config. at C-3	IC_{50} (nM)
27a	1	*S*	11
6	2	*S*	94
27b	3	*S*	130
27c	2	*R*	46

5. Computational docking study

Recently, Thoma et al. have reported a crystal structure of human DPP-IV (residues 31–766) expressed in the yeast *Pichia pastoris* and also that of DPP-IV–diprotin A (Ile-Pro-Ile) complex.[21] The computational docking simulation usually provides a reasonable explanation for the observed structure–activity relationship of the enzyme inhibitory activity. Therefore, we carried out the docking simulation of the human DPP-IV (Protein Data Bank, code 1NU8) with sulphostin to examine the interactions of the functional groups on the piperidine ring with DPP-IV, using Insight II software (Accelrys) on the Silicon Graphics workstation. The reported single-crystal structure[12] of sulphostin was inserted into the active site of DPP-IV instead of diprotin A. In this case, the positional relations between the C-2 carbonyl group and the N-1 phosphinyl group were fixed, and the direction of sulfonic acid group was slightly adjusted for stabilization of the complex. The side chain of amino acid residues of DPP-IV was also similarly adjusted. The result of the computational docking simulation is shown in Figure 3. The docking simulation suggested that sulphostin reasonably fitted into the active site of the enzyme surface. The phosphorus atom of the amino(sulfoamino)phosphinyl group was positioned in the vicinity of the hydroxyl group of ^{630}Ser of DPP-IV.

The C-3 amino group of sulphostin made ionic bonds with both ^{206}Glu and ^{205}Glu of DPP-IV those made ionic bonds with the N-terminal amino group of diprotin A.[21] The C-2 carbonyl group formed a hydrogen bond with ^{662}Tyr. The oxygen atom of the phosphinyl group at N-1 formed hydrogen bonds with ^{547}Tyr, which stabilized the oxyanion of the tetrahedral

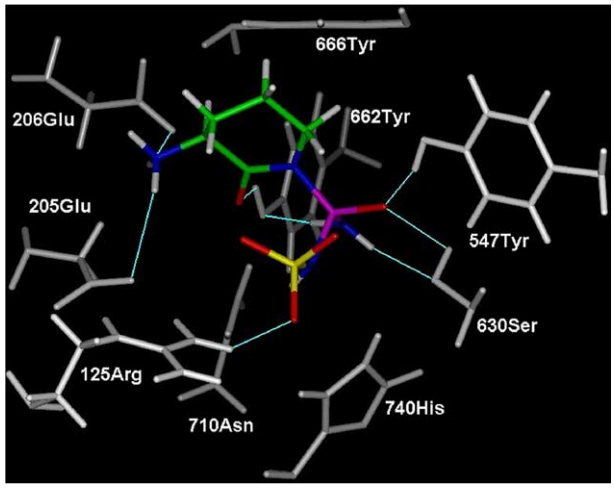


Figure 3. Docking model between DPP-IV and sulphostin (**1**). The side chain of the amino acid residues of DPP-IV, which existed the distance less than 3.0 Å from sulphostin (**1**), was selected and shown with white bold lines. Sulphostin was shown with color bold lines, and its carbon atoms were shown in green, hydrogens were white, nitrogens were blue, oxygens were red, phosphorus was light purple, and sulfur was yellow. The intermolecular hydrogen bonds were shown with light blue lines.

intermediate of diprotin A,[21] and with ^{630}Ser. The primary amino group of the amino(sulfoamino)phosphinyl group similarly formed hydrogen bonds with both ^{630}Ser and ^{662}Tyr. Moreover, the sulfonic acid group made an ionic bond with ^{125}Arg that made ionic bonds with the C-terminal carboxylic acid group of diprotin A.[21] Thus, it was confirmed that all functional groups of sulphostin interacted with DPP-IV. The C-3 amino group, the N-1 phosphinyl group, and the sulfonic acid group of sulphostin were correspondent with the N-terminal amino group, the carbonyl group of the central residual proline, and the C-terminal carboxylic acid group of diprotin A, respectively. It was therefore considered that the structure of sulphostin was a Gly-Pro-Gly tripeptide mimic, and that of the sulfonic acid-deficient analogue **6**, which was a minimum unit to exhibit the DPP-IV inhibitory activity, was a Gly-Pro dipeptide mimic. The results of the present analogue study concerning the functional groups could be explained without contradiction by the computational docking study.

6. Conclusion

To understand the required partial structure of sulphostin for the DPP-IV inhibitory activity, various analogues, namely the functional group-deficient analogues, the position-changed analogues, and the ring size-changed analogues were synthesized, and their DPP-IV inhibitory activities were examined. Moreover, the computational docking simulation between DPP-IV and sulphostin was carried out using the reported three-dimensional structure of the enzyme. As a result, it was confirmed that sulphostin interacted with DPP-IV similar fashion to tripeptide analogues such as Gly-Pro-Gly, although it was unique in appearance. The structure of sulphostin is very compact, and all of its functional groups are essential to the stable expression of the DPP-IV inhibitory activity. The sulfonic acid-deficient analogues, of which the syntheses were very facile compared to sulphostin, also had strong DPP-IV inhibitory activities. These analogues are expected to be effective therapeutic agents for type 2 diabetes and/or immune-related disorders.

7. Experimental

7.1. General experimental methods

The melting points were determined with Yanagimoto micro melting point apparatus. Optical rotations were measured with Perkin–Elmer Model 241 polarimeter. ^{1}H, ^{13}C, and ^{31}P NMR spectra were recorded with a JEOL GX-400 spectrometer, a JEOL JNM-A500 spectrometer, or a JEOL JNM-ECA600 spectrometer. TMS or 3-(trimethylsilyl)propionic-2,2,3,3-d_4 acid sodium salt was used as an internal standard substance for ^{1}H and ^{13}C NMR spectra, and 85% H_3PO_4 was used as an external standard substance for ^{31}P NMR spectrum. HR-ESIMS spectra were measured with a JEOL JMS-T100LC.

7.2. (3*S*)-3-Amino-2-piperidinone hydrochloride (4)

To a solution of (3*S*)-3-benzyloxycarbonylamino-2-piperidinone (**3**) (2.48 g, 9.99 mmol) in MeOH (20.0 mL) was added Pd-black (0.10 g), and the suspension was stirred for 20 h at room temperature under a hydrogen atmosphere. The reaction mixture was filtrated to remove Pd-black. After addition of 1.0 N HCl(aq) (10.0 mL, 10.0 mmol) to the filtrate, the mixture was evaporated under reduced pressure. The residue was crystallized by trituration in Et_2O to give compound **4** (1.39 g) in 93% yield as a colorless powder: ^{1}H NMR (400 MHz, D_2O) δ 3.99 (1H, dd, J = 11.2, 6.3 Hz, H-3), 3.35 (2H, td, J = 3.9, 5.9 Hz, H-6), 2.33 (1H, m, H-4a), 2.03 (1H, m, H-5a), 1.82–1.97 (2H, m, H-4b and H-5b); ^{13}C NMR (100 MHz, D_2O) δ 171.7 (C-2), 52.3 (C-3), 44.2 (C-6), 27.5 (C-4), 22.7 (C-5); HR-ESIMS m/z calcd for $C_5H_{11}N_2O$ $(M+H)^+$ 115.0871, found 115.0867.

7.3. (3*S*)-3-Amino-1-diaminophosphinyl-2-piperidinone 1/2 (+)-dibenzoyl-D-tartarate (6)

To a solution of (3*S*)-3-benzyloxycarbonylamino-1-diamino-phosphinyl-2-piperidinone (**5**) (1.00 g, 3.06 mmol) in EtOH (10.0 mL) and water (2.0 mL) was added Pd-black (0.050 g), and the suspension was stirred for 6 h at room temperature under a hydrogen atmosphere. The reaction mixture was filtrated to remove Pd-black, and the filtrate was diluted with EtOH (10 mL). To the solution was added a solution of (+)-dibenzoyl-D-tartaric acid (0.58 g, 1.53 mmol) in EtOH (6.0 mL), followed by addition of EtOH (40 mL). The precipitate was collected by filtration and was washed with EtOH to give compound **6** (0.99 g) in 87% yield as a colorless powder: ^{1}H NMR (400 MHz, D_2O) δ 8.13 (2H, dd, J = 7.8, 1.5 Hz, CO*Ph*), 7.72 (1H, tt, J = 7.8, 1.5 Hz, CO*Ph*), 7.57 (2H, t, J = 7.8 Hz, CO*Ph*), 5.72 (1H, s, C*H*(OBz)-COO), 4.10 (1H, dd, J = 11.7, 6.8 Hz, H-3), 3.65 (2H, td, J = 6.4, 3.9 Hz, H-6), 2.37 (1H, tdd, J = 5.4, 12.2, 6.4 Hz, H-4a), 1.89–2.07 (2H, m, H-5), 1.85 (1H, tdd, J = 12.2, 9.8, 6.4 Hz, H-4b); ^{13}C NMR (100 MHz, D_2O) δ 175.9 (CH*CO*O), 174.7 (C-2), 170.7 (Ph*CO*O), 136.9 (Ph), 132.7 (Ph × 2), 131.8 (Ph), 131.6 (Ph × 2), 78.1 (*C*H(OBz)COO), 53.3 (C-3), 47.3 (C-6), 26.6 (C-4), 23.1 (C-5); ^{31}P NMR (243 MHz, D_2O) δ 17.35; HR-ESIMS m/z calcd for $C_5H_{13}N_4NaO_2P$ $(M+Na)^+$ 215.0674, found 215.0687.

7.4. 1-Diaminophosphinyl-2-piperidinone (8)

To a solution of 2-piperidinone (**7**) (3.00 g, 30.3 mmol) in anhydrous THF (80 mL) was slowly added 1.54 M *n*-butyllithium solution in *n*-hexane (19.7 mL, 30.3 mmol) at −78 °C under a nitrogen atmosphere, and the mixture was stirred for 1 h. To the mixture was added a solution of phosphoryl chloride (5.10 g, 33.3 mmol) in anhydrous THF (15 mL) at −78 °C, and the mixture was stirred for 2 h. After addition of liquid NH_3 (3.30 mL, 140.0 mmol), the mixture was diluted with saturated NaCl(aq) (150 mL), and then water was added to the solution until the aqueous layer turned clear. The aqueous solution was washed with *n*-hexane (100 mL) and was purified

by Diaion® HP-20SS column chromatography eluted with water–20% MeOH/water. The solid residue was recrystallized from MeOH–EtOH to give compound **8** (1.78 g) in 33% yield as a colorless powder: ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.14 (4H, br s, $PNH_2 \times 2$), 3.44–3.50 (2H, m, H-6), 2.28 (2H, t, $J = 6.8$ Hz, H-3), 1.63–1.73 (4H, m, H-4 and H-5); ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.3 (C-2), 44.0 (C-6), 32.7 (C-3), 22.3 (C-5), 20.0 (C-4); HR-ESIMS *m/z* calcd for $C_5H_{12}N_3NaO_2P$ $(M+Na)^+$ 200.0565, found 200.0565.

7.5. (RS_P)-1-Amino(sulfoamino)phosphinyl-2-piperidinone sodium salt (9)

To a suspension of compound **8** (700.8 mg, 3.96 mmol) in DMF (10.0 mL) was added SO_3·pyridine complex (944.6 mg, 5.93 mmol) at 0 °C, and the suspension was stirred for 2 h at 6–8 °C. The reaction mixture was diluted with water (100 mL) and was passed through a Dowex® 50W × 8 (Na form) column. The eluate was washed with $CHCl_3$, and then benzylamine (1.30 mL, 11.90 mmol) and 1 N HCl(aq) (11.5 mL, 11.50 mmol) were added. The mixture was purified by Diaion® HP-20SS column chromatography eluted with water–70% MeOH/water. The residue was passed through a Dowex® 50W × 8 (Na form) column, and the eluate was evaporated under reduced pressure. The residue was crystallized from water–EtOH to give compound **9** (450.5 mg) in 41% yield as a colorless powder: ^{1}H NMR (400 MHz, D_2O) δ 3.67 (1H, m, H-6a), 3.57 (1H, m, H-6b), 2.44–2.50 (2H, m, H-3), 1.75–1.85 (4H, m, H-4 and H-5); ^{13}C NMR (100 MHz, D_2O) δ 181.2 (C-2), 48.8 (C-6), 35.5 (C-3), 24.8 (C-5), 22.3 (C-4); ^{31}P NMR (243 MHz, D_2O) δ 6.88; HR-ESIMS *m/z* calcd for $C_5H_{11}N_3O_5PS$ $(M-Na)^-$ 256.0157, found 256.0155.

7.6. (3*S*)-3-Benzyloxycarbonylaminopiperidine hydrochloride (10)

To a solution of (3*S*)-3-benzyloxycarbonylamino-2-piperidinone (**3**) (1.00 g, 4.03 mmol) in toluene (60 mL) was added 1.01 M diisobutylaluminum hydride solution in toluene (8.9 mL, 8.99 mmol) at −78 °C. The mixture was stirred for 1.5 h at −78 °C and then 1 h at 0 °C. Moreover, 1.01 M diisobutylaluminum hydride solution in toluene (2.0 mL, 2.02 mmol) was added, and the mixture was stirred for 30 min at 0 °C. After addition of 2 N HCl(aq) (20 mL, 40 mmol) at 0 °C, the solution was separated. The aqueous layer was purified by Diaion® HP-20SS column chromatography eluted with water–20% MeOH/water to give compound **10** (0.39 g) in 36% yield as a colorless oil: ^{1}H NMR (400 MHz, D_2O) δ 7.37–7.49 (5H, m, Ph), 5.12 (2H, br s, OCH_2Ph), 3.80 (1H, m, H-3), 3.41 (1H, dd, $J = 12.7$, 3.9 Hz, H-2a), 3.31 (1H, m, H-6a), 2.98 (1H, m, H-6b), 2.90 (1H, dd, $J = 12.7$, 10.3 Hz, H-2b), 1.95–2.07 (2H, m, H-4a and H-5a), 1.78 (1H, m, H-5b), 1.57 (1H, m, H-4b); ^{13}C NMR (100 MHz, D_2O) δ 160.4 (NHCOO), 139.2 (Ph), 131.7 (Ph × 2), 131.3 (Ph), 130.6 (Ph × 2), 70.0 (OCH_2Ph), 49.5 (C-2), 47.9 (C-3), 46.4 (C-6), 30.4 (C-4), 23.2 (C-5); HR-ESIMS *m/z* calcd for $C_{13}H_{19}N_2O_2$ $(M+H)^+$ 235.1447, found 235.1451.

7.7. (3*S*)-3-Benzyloxycarbonylamino-1-diaminophosphinylpiperidine (11)

To a solution of compound **10** (1.16 g, 4.28 mmol) in anhydrous THF (20.0 mL) were added triethylamine (1.80 mL, 12.9 mmol) and a solution of phosphoryl chloride (0.79 g, 5.15 mmol) in anhydrous THF (30.0 mL) at 0 °C, and the mixture was stirred for 30 min. To the mixture was added 2.2 M NH_3 (10.0 mL, 22.0 mmol) solution in $CHCl_3$, which was prepared from liquid NH_3 (5.0 mL) and $CHCl_3$ (100 mL), at 0 °C, and the mixture was stirred for 5 min. The reaction mixture was evaporated under reduced pressure. The residue was purified by Diaion® HP-20SS column chromatography eluted with water–60% MeOH/water and then silica gel column chromatography ($CHCl_3$–MeOH = 14:1–9:1) to give compound **11** (0.76 g) in 57% yield as a colorless powder: ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.28–7.40 (5H, m, Ph), 7.21 (1H, br d, $J = 7.8$ Hz, CH*NH*COO), 5.01 (2H, br s, OCH_2Ph), 3.54 (2H, br s, PNH_2), 3.53 (2H, br s, PNH_2), 3.32–3.46 (2H, m, H-2a and H-3), 3.24 (1H, m, H-6a), 2.50 (1H, m, H-6b), 2.38 (1H, m, H-2b), 1.76 (1H, m, H-4a), 1.56 (1H, m, H-5a), 1.38 (1H, m, H-5b), 1.27 (1H, m, H-4b); ^{13}C NMR (100 MHz, DMSO-d_6) δ 155.3 (NHCOO), 137.2 (Ph), 128.3 (Ph × 3), 127.8 (Ph × 2), 65.1 (OCH_2Ph), 49.5 (C-2), 47.2 (C-3), 44.4 (C-6), 30.7 (C-4), 24.1 (C-5); HR-ESIMS *m/z* calcd for $C_{13}H_{21}N_4NaO_3P$ $(M+Na)^+$ 335.1249, found 335.1242.

7.8. (3*S*,RS_P)-1-Amino(sulfoamino)phosphinyl-3-benzyloxycarbonylamino piperidine sodium salt (12)

To a solution of compound **11** (156.2 mg, 0.500 mmol) in DMF (2.0 mL) was added SO_3·pyridine complex (95.5 mg, 0.600 mmol) at 0 °C, and the mixture was stirred for 5 h at 6–8 °C. After addition of water (40 mL) and $NaHCO_3$ (126.0 mg, 1.500 mmol), the reaction mixture was purified by Diaion® HP-20SS column chromatography eluted with water–30% MeOH/water to give compound **12** (97.3 mg) in 47% yield as a colorless powder: ^{1}H NMR (400 MHz, D_2O) δ 7.37–7.47 (5H, m, Ph), 5.12 (2H, br s, OCH_2Ph), 3.59 (1H, m, H-3), 3.39 (1H, m, H-2a), 3.20 (1H, m, H-6a), 2.85–3.10 (2H, m, H-2b and H-6b), 1.85 (1H, m, H-4a), 1.71 (1H, m, H-5a), 1.45–1.60 (2H, m, H-4b and H-5b); ^{13}C NMR (100 MHz, D_2O) δ 160.6 (NHCOO), 139.4 (Ph), 131.7 (Ph × 2), 131.2 (Ph), 130.5 (Ph × 2), 69.6 (OCH_2Ph), 52.0 and 51.6 (C-2), 49.6 and 49.4 (C-3), 47.6 and 47.3 (C-6), 32.3 and 32.1 (C-4), 25.8 and 25.4 (C-5); HR-ESIMS *m/z* calcd for $C_{13}H_{20}N_4O_6PS$ $(M-Na)^-$ 391.0841, found 391.0818.

7.9. (3*S*,RS_P)-3-Amino-1-amino(sulfoamino)phosphinylpiperidine (13)

A solution of benzylamine hydrochloride (125.0 mg, 0.870 mmol) and compound **12** (180.0 mg, 0.434 mmol) in water (5 mL) was purified by Diaion® HP-20SS column chromatography eluted with water–70% MeOH/water to give the sulfonic acid benzylamine salt (193.0 mg) as a colorless oil. To a solution of the benzylamine salt (193.0 mg, 0.306 mmol) in EtOH (2.0 mL) and

water (0.5 mL) was added Pd-black (20.0 mg), and the suspension was stirred for 15 h at room temperature under a hydrogen atmosphere. The reaction mixture was filtrated, and the filtrate was evaporated under reduced pressure. The residue was purified by Diaion® HP-20SS column chromatography eluted with water to give compound **13** (88.8 mg) in 79% yield as a colorless powder: ^{1}H NMR (400 MHz, D_2O) δ 3.41–3.55 (2H, m, H-2a and H-3), 3.11–3.31 (3H, m, H-2b and H-6), 2.03 (1H, m, H-4a), 1.70–1.84 (2H, m, H-4b and H-5a), 1.64 (1H, m, H-5b); ^{13}C NMR (100 MHz, D_2O) δ 49.9 and 49.4 (C-2), 49.7 and 49.6 (C-3), 47.3 and 47.2 (C-6), 30.1 and 29.9 (C-4), 24.2 and 23.9 (C-5); ^{31}P NMR (202 MHz, D_2O) δ 13.03 and 12.68; HR-ESIMS *m/z* calcd for $C_5H_{14}N_4O_4PS$ $(M-H)^-$ 257.0473, found 257.0493.

7.10. (5*S*)-5-Benzyloxycarbonylamino-1-diaminophosphinyl-2-piperidinone (15)

To a solution of (5*S*)-5-benzyloxycarbonylamino-2-piperidinone (**14**) (2.48 g, 9.99 mmol) in anhydrous THF (50 mL) was slowly added 1.54 M *n*-butyllithium solution in *n*-hexane (6.50 mL, 10.0 mmol) at −78 °C under a nitrogen atmosphere, and the mixture was stirred for 20 min. To the mixture was added a solution of phosphoryl chloride (1.53 g, 9.98 mmol) in anhydrous THF (5.0 mL) at −78 °C, and the mixture was stirred for 1.5 h. After addition of liquid NH_3 (1.00 mL, 45.3 mmol), the mixture was diluted with saturated NaCl(aq) (100 mL), and then water was added until the aqueous solution turned clear. The aqueous solution was washed with *n*-hexane (50 mL) and was purified by Diaion® HP-20 column chromatography eluted with water–75% MeOH/water. The solid residue was washed with MeOH–Et_2O to give compound **15** (2.00 g) in 61% yield as a colorless powder: ^{1}H NMR (400 MHz, D_2O) δ 7.38–7.50 (5H, m, Ph), 5.14 (2H, br s, O*CH*$_2$Ph), 4.00 (1H, m, H-5), 3.75 (1H, td, J = 12.7, 3.9 Hz, H-6a), 3.53 (1H, ddd, J = 12.7, 6.8, 3.4 Hz, H-6b), 2.56 (2H, t, J = 6.8 Hz, H-3), 2.10 (1H, m, H-4a), 1.84 (1H, m, H-4b); ^{13}C NMR (100 MHz, D_2O) δ 180.3 (C-2), 160.6 (NH*COO*), 139.3 (Ph), 131.7 (Ph × 2), 131.3 (Ph), 130.6 (Ph × 2), 69.8 (O*C*H$_2$Ph), 51.2 (C-5), 47.7 (C-6), 32.8 (C-3), 27.7 (C-4); HR-ESIMS *m/z* calcd for $C_{13}H_{19}N_4NaO_4P$ $(M+Na)^+$ 349.1042, found 349.1045.

7.11. (5*S*,*S* or R_P)-1-Amino(sulfoamino)phosphinyl-5-benzyloxycarbonylamino-2-piperidinone sodium salt (16: more polar compound by reversed-phase HPLC) and (5*S*,*R* or S_P)-1-amino(sulfoamino)phosphinyl-5-benzyloxycarbonylamino-2-piperidinone sodium salt (17: less polar compound by reversed-phase HPLC)

To a solution of compound **15** (1.63 g, 5.00 mmol) in DMF (16.3 mL) was added SO_3·pyridine complex (1.19 g, 7.48 mmol) at 0 °C, and the mixture was stirred for 4 h at 6–8 °C. After addition of water (100 mL) and $NaHCO_3$ (1.26 g, 15.0 mmol), the reaction mixture was purified[22] by Diaion® HP-20SS column chromatography eluted with water–30% MeOH/water to give compounds **16** (0.38 g) and **17** (0.34 g) in 18% and 16% yield as a colorless powder, respectively.

7.11.1. Physicochemical data of compound 16. $[\alpha]_D^{23}$ −19.4 (*c* 1.0, H_2O); ^{1}H NMR (400 MHz, D_2O) δ 7.38–7.48 (5H, m, Ph), 5.08–5.18 (2H, m, O*CH*$_2$Ph), 4.01 (1H, m, H-5), 3.84 (1H, td, J = 3.9, 12.7 Hz, H-6a), 3.59 (1H, m, H-6b), 2.49 (2H, t, J = 7.3 Hz, H-3), 2.07 (1H, m, H-4a), 1.84 (1H, m, H-4b); ^{13}C NMR (100 MHz, D_2O) δ 179.6 (C-2), 160.7 (NHCOO), 139.4 (Ph), 131.7 (Ph × 2), 131.2 (Ph), 130.5 (Ph × 2), 69.7 (O*CH*$_2$Ph), 51.8 (C-5), 47.7 (C-6), 32.8 (C-3), 28.0 (C-4); ^{31}P NMR (202 MHz, D_2O) δ 5.09; HR-ESIMS *m/z* calcd for $C_{13}H_{18}N_4O_7PS$ $(M-Na)^-$ 405.0634, found 405.0604; reversed-phase HPLC analysis: retention time 14.2 min (conditions; column: Senshu Pak PEGASIL ODS (4.6 × 250 mm), eluent: 0.1% trifluoroacetic acid(-aq)–MeOH = 75:25, flow rate: 1.0 mL/min, detection: UV 205 nm, column temperature: 40 °C).

7.11.2. Physicochemical data of compound 17. $[\alpha]_D^{23}$ +13.8 (*c* 0.5, H_2O); ^{1}H NMR (400 MHz, D_2O) δ 7.38–7.48 (5H, m, Ph), 5.09–5.19 (2H, m, O*CH*$_2$Ph), 3.97 (1H, m, H-5), 3.69–3.74 (2H, m, H-6), 2.41–2.57 (2H, m, H-3), 2.03 (1H, m, H-4a), 1.91 (1H, m, H-4b); ^{13}C NMR (100 MHz, D_2O) δ 179.5 (C-2), 160.7 (NHCOO), 139.4 (Ph), 131.7 (Ph × 2), 131.2 (Ph), 130.5 (Ph × 2), 69.7 (O*CH*$_2$Ph), 51.5 (C-5), 47.7 (C-6), 32.6 (C-3), 27.9 (C-4); ^{31}P NMR (202 MHz, D_2O) δ 5.22; HR-ESIMS *m/z* calcd for $C_{13}H_{18}N_4O_7PS$ $(M-Na)^-$ 405.0634, found 405.0612; reversed-phase HPLC analysis: retention time 15.3 min (conditions were similar to those noted for compound **16**).

7.12. (5*S*,*S* or R_P)-5-Amino-1-amino(sulfoamino)phosphinyl-2-piperidinone (18)

A solution of benzylamine hydrochloride (125.7 mg, 0.875 mmol) and compound **16** (250.0 mg, 0.584 mmol) in water (2 mL) was purified by Diaion® HP-20SS column chromatography eluted with water–70% MeOH/water to give the sulfonic acid benzylamine salt (217.6 mg) as a colorless oil. To a solution of the benzylamine salt (211.8 mg, 0.401 mmol) in MeOH (6.0 mL) and water (3.0 mL) was added Pd-black (43.0 mg), and the suspension was stirred for 4 h at room temperature under a hydrogen atmosphere. The reaction mixture was filtrated, and the filtrate was evaporated under reduced pressure. The residue was purified by Diaion® HP-20SS column chromatography eluted with water. The solid residue was recrystallized from water–EtOH to give compound **18** (95.1 mg) in 58% yield as a colorless powder: ^{1}H NMR (400 MHz, D_2O) δ 4.04 (1H, m, H-6a), 3.85 (1H, m, H-5), 3.70 (1H, ddd, J = 13.2, 7.8, 3.4 Hz, H-6b), 2.61–2.67 (2H, m, H-3), 2.35 (1H, m, H-4a), 1.98 (1H, m, H-4b); ^{13}C NMR (100 MHz, D_2O) δ 179.0 (C-2), 49.3 (C-5), 47.7 (C-6), 32.6 (C-3), 26.3 (C-4); ^{31}P NMR (202 MHz, D_2O) δ 6.67; HR-ESIMS *m/z* calcd for $C_5H_{12}N_4O_5PS$ $(M-H)^-$ 271.0266, found 271.0254.

7.13. (5*S*,*R* or S_P)-5-Amino-1-amino(sulfoamino)phosphinyl-2-piperidinone (19)

The synthetic method similar to that noted for compound **18** was used. A mixture of benzylamine hydro-

chloride (140.0 mg, 0.975 mmol) and compound **17** (140.0 mg, 0.327 mmol) was purified to give the sulfonic acid benzylamine salt (121.6 mg) as a colorless oil. The benzylamine salt (118.0 mg, 0.230 mmol) was treated with Pd-black (20.0 mg) under a hydrogen atmosphere, followed by purification using Diaion® HP-20SS column chromatography eluted with water and then recrystallization from water–EtOH to give compound **19** (57.8 mg) in 63% yield as a colorless powder: ^{1}H NMR (400 MHz, D_2O) δ 3.83–3.99 (3H, m, H-5 and H-6), 2.58–2.64 (2H, m, H-3), 2.40 (1H, m, H-4a), 1.97 (1H, m, H-4b); ^{13}C NMR (100 MHz, D_2O) δ 179.5 (C-2), 49.0 (C-5), 47.8 (C-6), 32.2 (C-3), 26.0 (C-4); ^{31}P NMR (202 MHz, D_2O) δ 6.25; HR-ESIMS *m/z* calcd for $C_5H_{12}N_4O_5PS$ $(M-H)^-$ 271.0266, found 271.0240.

7.14. N^{α}-Benzyloxycarbonyl-L-glutamine methyl ester

To a solution of N^{α}-benzyloxycarbonyl-L-glutamine (8.00 g, 28.5 mmol) in DMF (80.0 mL) were added $NaHCO_3$ (4.80 g, 57.1 mmol) and iodomethane (4.44 mL, 71.3 mmol), and the mixture was stirred for 18 h at room temperature. The reaction mixture was diluted with water and was extracted with EtOAc. The organic layer was washed with 10% $Na_2S_2O_3{\cdot}5H_2O$(aq) and saturated NaCl(aq). The resultant organic layer was dried over anhydrous Na_2SO_4 and was evaporated under reduced pressure. The solid residue was washed with EtOAc–Et_2O to give the target compound (6.60 g) in 79% yield as a colorless powder: mp 142–143 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75 (1H, br d, J = 7.3 Hz, CH*NH*COO), 7.29–7.41 (5H, m, Ph), 7.27 (1H, br s, CH_2CONHa), 6.78 (1H, br s, CH_2CONHb), 5.03 (2H, s, O*CH*$_2$Ph), 4.04 (1H, m, *CH*COOMe), 3.63 (3H, s, OMe), 2.16 (2H, t, J = 7.3 Hz, *CH*$_2$CONH$_2$), 1.95 (1H, m, CH_2CHaCH), 1.74 (1H, m, CH_2CHbCH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.2 (CH_2COO or $CONH_2$), 172.6 ($CONH_2$ or CH_2COO), 156.0 (NHCOO), 136.8 (Ph), 128.3 (Ph × 2), 127.7 (Ph), 127.6 (Ph × 2), 65.4 (O*CH*$_2$Ph), 53.4 (N*C*HCOO), 51.7 (OMe), 30.9 (NH_2CO*CH*$_2$), 26.3 (CH_2*CH*$_2$CH); HR-ESIMS *m/z* calcd for $C_{14}H_{18}N_2NaO_5$ $(M+Na)^+$ 317.1113, found 317.1119.

7.15. (3*S*)-3-Benzyloxycarbonylamino-2-pyrrolidinone (20a)

To a suspension of N^{α}-benzyloxycarbonyl-L-glutamine methyl ester (20.00 g, 68.0 mmol) and pyridine (12.1 mL, 153.0 mmol) in MeCN (130 mL) and water (130 mL) was added iodobenzene diacetate (24.08 g, 74.8 mmol), and the mixture was stirred for 2 h at room temperature. The reaction mixture was evaporated under reduced pressure to remove MeCN, and the remaining solution was washed with EtOAc. To the aqueous solution were added $NaHCO_3$ (17.13 g, 203.9 mmol) and $CHCl_3$ (100 mL), and the mixture was stirred for 2 h at room temperature. The reaction mixture was separated, and the aqueous layer was extracted with $CHCl_3$. The combined organic layer was washed with 10% $Na_2S_2O_3{\cdot}5H_2O$(aq), water, and saturated NaCl(aq). The resultant organic layer was then dried over anhydrous Na_2SO_4 and was evaporated under reduced pressure. The residue was purified by silica gel column chromatography ($CHCl_3$–MeOH = 39:1–14:1). The solid residue was washed with EtOAc–Et_2O to give compound **20a** (7.34 g) in 46% yield as a colorless powder:[23] mp 179–181 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28–7.38 (5H, m, Ph), 6.64 (1H, br s, H-1), 5.53 (1H, br d, J = 5.9 Hz, CH*NH*COO), 5.11 (2H, s, O*CH*$_2$Ph), 4.24 (1H, m, H-3), 3.33 (2H, m, H-5), 2.68 (1H, m, H-4a), 1.97 (1H, qd, J = 9.8, 12.2 Hz, H-4b); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.4 (C-2), 156.4 (NHCOO), 136.2 (Ph), 128.5 (Ph × 2), 128.2 (Ph), 128.1 (Ph × 2), 67.0 (O*C*H$_2$Ph), 51.9 (C-3), 39.0 (C-5), 30.0 (C-4); HR-ESIMS *m/z* calcd for $C_{12}H_{14}N_2NaO_3$ $(M+Na)^+$ 257.0902, found 257.0900.

7.16. (3*S*)-3-Benzyloxycarbonylamino-2-perhydroazepinone (20b)

To a solution of (3*S*)-3-amino-2-perhydroazepinone hydrochloride (5.00 g, 39.0 mmol) in THF (20.0 mL) and water (40.0 mL) were added $NaHCO_3$ (6.56 g, 78.0 mmol) and benzyl chloroformate (5.57 mL, 39.0 mmol) at 0 °C, and the mixture was stirred for 18 h at room temperature. The reaction mixture was separated, and the aqueous layer was extracted with EtOAc. The combined organic layer was washed with saturated NaCl(aq), dried over anhydrous Na_2SO_4, and evaporated under reduced pressure. The solid residue was washed with EtOAc–Et_2O to give compound **20b** (11.93 g) in 75% yield as a colorless powder: mp 125–127 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28–7.40 (5H, m, Ph), 6.47 (1H, br s, H-1), 6.19 (1H, br d, J = 4.9 Hz, CH*NH*COO), 5.06–5.15 (2H, m, O*CH*$_2$Ph), 4.34 (1H, m, H-3), 3.17–3.31 (2H, m, H-7), 2.11 (1H, m, H-4a), 2.01 (1H, m, H-5a), 1.71–1.88 (2H, m, H-6a, H-5b), 1.53 (1H, m, H-4b), 1.38 (1H, m, H-6b); ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.4 (C-2), 155.5 (NHCOO), 136.6 (Ph), 128.5 (Ph × 2), 128.03 (Ph), 127.98 (Ph × 2), 66.6 (O*C*H$_2$Ph), 53.7 (C-3), 42.1 (C-7), 32.0 (C-4), 28.9 (C-6), 28.0 (C-5); HR-ESIMS *m/z* calcd for $C_{14}H_{18}N_2NaO_3$ $(M+Na)^+$ 285.1215, found 285.1209.

7.17. (3*S*)-3-Benzyloxycarbonylamino-1-diaminophosphinyl-2-pyrrolidinone (21a)

The synthetic method similar to that noted for compound **15** was used. Compound **20a** (3.00 g, 12.8 mmol) was treated with 1.54 M *n*-butyllithium solution in *n*-hexane (7.90 mL, 12.2 mmol), phosphoryl chloride (1.96 g, 12.8 mmol), and liquid NH_3 (1.40 mL, 59.6 mmol), followed by purification using Diaion® HP-20SS column chromatography eluted with water–80% MeOH/water to give compound **21a** (1.83 g) in 46% yield as a colorless powder: mp 144–147 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.64 (1H, br d, J = 8.8 Hz, NHCOO), 7.28–7.40 (5H, m, Ph), 5.05 (2H, s, O*CH*$_2$Ph), 4.32 (2H, br s, PNH_2), 4.19–4.29 (3H, m, H-3 and PNH_2), 3.53 (1H, m, H-5a), 3.37 (1H, dd, J = 10.2, 6.4 Hz, H-5b), 2.23 (1H, m, H-4a), 1.83 (1H, m, H-4b); ^{13}C NMR (100 MHz, DMSO-d_6) δ 174.8 (C-2), 155.9 (NHCOO), 136.9 (Ph), 128.3 (Ph × 2), 127.74 (Ph), 127.71 (Ph × 2), 65.4 (O*C*H$_2$Ph), 53.3 (C-3), 42.1

(C-5), 26.2 (C-4); HR-ESIMS *m/z* calcd for $C_{12}H_{17}N_4NaO_4P$ $(M+Na)^+$ 335.0885, found 335.0882.

7.18. (3*S*)-3-Benzyloxycarbonylamino-1-diaminophosphinyl-2-perhydroazepinone (21b)

The synthetic method similar to that noted for compound **15** was used. Compound **20b** (4.00 g, 15.2 mmol) was treated with 1.55 M *n*-butyllithium solution in *n*-hexane (8.90 mL, 13.8 mmol), phosphoryl chloride (2.57 g, 16.8 mmol), and liquid NH_3 (2.00 mL, 90.5 mmol), followed by purification using silica gel column chromatography ($CHCl_3$–MeOH = 29:1–14:1) to give compound **21b** (1.55 g) in 30% yield as a colorless powder: mp 145–148 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.26–7.39 (6H, m, Ph and NHCOO), 4.97–5.09 (2H, m, OCH_2Ph), 4.43 (1H, t, J = 8.8 Hz, H-3), 4.06–4.25 (5H, m, $PNH_2 \times 2$ and H-7a), 3.26 (1H, m, H-7b), 1.59–1.85 (4H, m, H-5, H-4a and H-6a), 1.54 (1H, m, H-4b), 1.39 (1H, m, H-6b); ^{13}C NMR (100 MHz, DMSO-d_6) δ 175.7 (C-2), 155.3 (NHCOO), 137.0 (Ph), 128.2 (Ph × 2), 127.7 (Ph), 127.6 (Ph × 2), 65.2 (OCH_2Ph), 54.1 (C-3), 42.9 (C-7), 30.4 (C-4), 27.8 (C-6), 26.6 (C-5); HR-ESIMS *m/z* calcd for $C_{14}H_{21}N_4NaO_4P$ $(M+Na)^+$ 363.1198, found 363.1186.

7.19. (3*S*,R_P)-1-Amino(sulfoamino)phosphinyl-3-benzyloxycarbonylamino-2-pyrrolidinone sodium salt (22a) and (3*S*,S_P)-1-amino(sulfoamino)phosphinyl-3-benzyloxycarbonylamino-2-pyrrolidinone sodium salt (23a)

The synthetic method similar to that noted for compounds **16** and **17** was used. Compound **21a** (2.00 g, 6.4 mmol) was treated with SO_3·pyridine complex (2.04 g, 12.8 mmol), followed by addition of $NaHCO_3$ (1.61 g, 19.2 mmol). The separation[22] of the resultant diastereomers using Diaion® HP-20SS column chromatography eluted with water–30% MeOH/water gave compounds **22a** (0.48 g) and **23a** (0.53 g) in 18% and 20% yield, respectively, as a colorless powder.

7.19.1. Physicochemical data of compound 22a. ^{1}H NMR (400 MHz, D_2O) δ 7.39–7.49 (5H, m, Ph), 5.11–5.21 (2H, m, PhCH_2O), 4.50 (1H, dd, J = 10.7, 9.3 Hz, H-3), 3.82 (1H, m, H-5a), 3.58 (1H, m, H-5b), 2.46 (1H, m, H-4a), 2.02 (1H, m, H-4b); ^{13}C NMR (100 MHz, D_2O) δ 180.7 (C-2), 161.0 (NHCOO), 139.2 (Ph), 131.7 (Ph × 2), 131.3 (Ph), 130.6 (Ph × 2), 70.1 (OCH_2Ph), 56.7 (C-3), 46.8 (C-5), 29.5 (C-4); ^{31}P NMR (202 MHz, D_2O) δ 3.29; HR-ESIMS *m/z* calcd for $C_{12}H_{16}N_4Na_2O_7PS$ $(M+Na)^+$ 437.0273, found 437.0246; reversed-phase HPLC analysis: retention time 13.5 min (conditions; column: Senshu Pak PEGASIL ODS (4.6 × 250 mm), eluent: 0.1% trifluoroacetic acid-(aq)–MeOH = 75:25, flow rate: 1.0 mL/min, detection: UV 205 nm, column temperature: 40 °C).

7.19.2. Physicochemical data of compound 23a. ^{1}H NMR (400 MHz, D_2O) δ 7.39–7.49 (5H, m, Ph), 5.16 (2H, s, PhCH_2O), 4.43 (1H, br t, J = 9.8 Hz, H-3), 3.77 (1H, m, H-5a), 3.66 (1H, m, H-5b), 2.48 (1H, m, H-4a), 2.09 (1H, m, H-4b); ^{13}C NMR (100 MHz, D_2O) δ 181.0 (C-2), 160.7 (NHCOO), 139.1 (Ph), 131.7 (Ph × 2), 131.3 (Ph), 130.6 (Ph × 2), 70.1 (OCH_2Ph), 56.7 (C-3), 46.8 (C-5), 29.0 (C-4); ^{31}P NMR (202 MHz, D_2O) δ 3.70; HR-ESIMS *m/z* calcd for $C_{12}H_{16}N_4Na_2O_7PS$ $(M+Na)^+$ 437.0273, found 437.0245; reversed-phase HPLC analysis: retention time 12.6 min (conditions were similar to those noted for compound **22a**).

7.20. (3*S*,R_P)-1-Amino(sulfoamino)phosphinyl-3-benzyloxycarbonylamino-2-perhydroazepinone sodium salt (22b) and (3*S*,S_P)-1-amino(sulfoamino)-phosphinyl-3-benzyloxycarbonylamino-2-perhydroazepinone sodium salt (23b)

The synthetic method similar to that noted for compounds **16** and **17** was used. Compound **21b** (800.4 mg, 2.35 mmol) was treated with SO_3·pyridine complex (561.5 mg, 3.53 mmol), followed by addition of $NaHCO_3$ (592.7 mg, 7.06 mmol). The separation[22] of the resultant diastereomers using Diaion® HP-20SS column chromatography eluted with water–40% MeOH/water gave compounds **22b** (80.4 mg) and **23b** (40.4 mg) in 6% and 3% yield, respectively, as a colorless powder.

7.20.1. Physicochemical data of compound 22b. ^{1}H NMR (400 MHz, D_2O) δ 7.39–7.49 (5H, m, Ph), 5.09–5.21 (2H, m, PhCH_2O), 4.57 (1H, m, H-3), 4.11 (1H, m, H-7a), 3.48 (1H, m, H-7b), 1.68–2.01 (6H, m, H-4, H-5, and H-6); ^{13}C NMR (100 MHz, D_2O) δ 181.3 (C-2), 160.5 (NHCOO), 139.3 (Ph), 131.7 (Ph × 2), 131.3 (Ph), 130.6 (Ph × 2), 70.0 (OCH_2Ph), 58.2 (C-3), 47.6 (C-7), 32.7 (C-4), 29.8 (C-5), 29.6 (C-6); ^{31}P NMR (202 MHz, D_2O) δ 7.72; HR-ESIMS *m/z* calcd for $C_{14}H_{20}N_4Na_2O_7PS$ $(M+Na)^+$ 465.0586, found 465.0631; reversed-phase HPLC analysis: retention time 10.5 min (conditions; column: Senshu Pak PEGASIL ODS (4.6 × 250 mm), eluent: 0.1% trifluoroacetic acid-(aq)–MeOH = 65:35, flow rate: 1.0 mL/min, detection: UV 205 nm, column temperature: 40 °C).

7.20.2. Physicochemical data of compound 23b. ^{1}H NMR (400 MHz, D_2O) δ 7.39–7.49 (5H, m, Ph), 5.10–5.20 (2H, m, PhCH_2O), 4.63 (1H, m, H-3), 4.13 (1H, m, H-7a), 3.46 (1H, m, H-7b), 1.87–1.99 (3H, m, H-4a, H-5a, and H-6a), 1.66–1.82 (2H, m, H-4b and H-5b), 1.46 (1H, m, H-6b); ^{13}C NMR (125 MHz, D_2O) δ 181.2 (C-2), 160.3 (NHCOO), 139.3 (Ph), 131.7 (Ph × 2), 131.3 (Ph), 130.6 (Ph × 2), 70.0 (OCH_2Ph), 57.8 (C-3), 47.8 (C-7), 33.6 (C-4), 30.7 (C-6), 29.5 (C-5); ^{31}P NMR (202 MHz, D_2O) δ 7.54; HR-ESIMS *m/z* calcd for $C_{14}H_{20}N_4Na_2O_7PS$ $(M+Na)^+$ 465.0586, found 465.0613; reversed-phase HPLC analysis: retention time 11.6 min (conditions were similar to those noted for compound **22b**).

7.21. (3*S*,R_P)-3-Amino-1-amino(sulfoamino)phosphinyl-2-pyrrolidinone (24a)

The synthetic method similar to that noted for compound **18** was used. A mixture of (*S*)-(−)-phenylethylamine hydrochloride (1.00 g, 6.34 mmol) and compound **22a** (0.48 g, 1.16 mmol) was purified by Diaion® HP-20SS column chromatography to give the sulfonic acid (*S*)-(−)-1-phenylethylamine salt (0.13 g) as a

colorless oil. The (*S*)-(−)-1-phenylethylamine salt (111.7 mg, 0.218 mmol) was treated with Pd-black (11.0 mg) under a hydrogen atmosphere, followed by purification using Diaion® HP-20SS column chromatography eluted with water and then recrystallization from water–EtOH to give compound **24a** (50.5 mg) in 20% yield as a colorless powder: $[\alpha]_D^{24}$ −47.6 (*c* 0.5, H_2O); ^1H NMR (500 MHz, D_2O) δ 4.30 (1H, dd, J = 11.8, 8.5 Hz, H-3), 3.91 (1H, m, H-5a), 3.71 (1H, td, J = 10.4, 6.3 Hz, H-5b), 2.65 (1H, m, H-4a), 2.21 (1H, m, H-4b); ^{13}C NMR (125 MHz, D_2O) δ 176.6 (C-2), 54.8 (C-3), 47.4 (C-5), 27.9 (C-4); ^{31}P NMR (202 MHz, D_2O) δ 2.89; HR-ESIMS *m*/*z* calcd for $C_4H_{10}N_4O_5PS$ $(M-H)^-$ 257.0110, found 257.0103.

7.22. (3*S*,S_P)-3-Amino-1-amino(sulfoamino)phosphinyl-2-pyrrolidinone (25a)

The synthetic method similar to that noted for compound **18** was used. A mixture of (*S*)-(−)-phenylethylamine hydrochloride (1.00 g, 6.34 mmol) and compound **23a** (0.53 g, 1.28 mmol) was purified by Diaion® HP-20SS column chromatography to give the sulfonic acid (*S*)-(−)-1-phenylethylamine salt (0.20 g) as a colorless oil. The (*S*)-(−)-1-phenylethylamine salt (179.5 mg, 0.345 mmol) was treated with Pd-black (18.0 mg) under a hydrogen atmosphere, followed by purification using Diaion® HP-20SS column chromatography eluted with water and then recrystallization from water–EtOH to give compound **25a** (73.0 mg) in 25% yield as a colorless powder: $[\alpha]_D^{24}$ +6.3 (*c* 0.5, H_2O); ^1H NMR (400 MHz, D_2O) δ 4.28 (1H, dd, J = 11.7, 8.8 Hz, H-3), 3.87 (1H, m, H-5a), 3.75 (1H, td, J = 10.3, 6.4 Hz, H-5b), 2.66 (1H, m, H-4a), 2.18 (1H, tdd, J = 11.7, 10.3, 9.3 Hz, H-4b); ^{13}C NMR (100 MHz, D_2O) δ 176.8 (C-2), 54.8 (C-3), 47.3 (C-5), 27.8 (C-4); ^{31}P NMR (202 MHz, D_2O) δ 2.98; HR-ESIMS *m*/*z* calcd for $C_4H_{10}N_4O_5PS$ $(M-H)^-$ 257.0110, found 257.0133.

7.23. (3*S*,R_P)-3-Amino-1-amino(sulfoamino)phosphinyl-2-perhydroazepinone (24b)

The synthetic method similar to that noted for compound **18** was used. A mixture of (*S*)-(−)-1-phenylethylamine hydrochloride (100.0 mg, 0.634 mmol) and compound **22b** (80.4 mg, 0.191 mmol) was purified by Diaion® HP-20SS column chromatography to give the sulfonic acid (*S*)-(−)-1-phenylethylamine salt (69.8 mg) as a colorless oil. The (*S*)-(−)-1-phenylethylamine salt (69.3 mg, 0.128 mmol) was treated with Pd-black (6.9 mg) under a hydrogen atmosphere, followed by purification using Diaion® HP-20SS chromatography eluted with water and then recrystallization from water–EtOH to give compound **24b** (22.0 mg) in 38% yield as a colorless powder: ^1H NMR (400 MHz, D_2O) δ 4.49 (1H, dd, J = 10.3, 3.4 Hz, H-3), 4.14 (1H, ddd, J = 15.6, 10.7, 5.9 Hz, H-7a), 3.40 (1H, td, J = 10.7, 15.6 Hz, H-7b), 1.96–2.10 (3H, m, H-4 and H-5a), 1.89 (1H, m, H-6a), 1.72–1.85 (2H, m, H-5b and H-6b); ^{13}C NMR (100 MHz, D_2O) δ 177.7 (C-2), 57.5 (C-3), 48.0 (C-7), 30.8 (C-4), 29.4 (C-5 and C-6); ^{31}P NMR (202 MHz, D_2O) δ 7.34; HR-ESIMS *m*/*z* calcd for $C_6H_{14}N_4O_5PS$ $(M-H)^-$ 285.0423, found 285.0403.

7.24. (3*S*,S_P)-3-Amino-1-amino(sulfoamino)phosphinyl-2-perhydroazepinone (25b)

The synthetic method similar to that noted for compound **18** was used. A mixture of (*S*)-(−)-1-phenylethylamine hydrochloride (100.0 mg, 0.634 mmol) and compound **23b** (40.4 mg, 0.096 mmol) was purified by Diaion® HP-20SS column chromatography to give the sulfonic acid (*S*)-(−)-1-phenylethylamine salt (30.0 mg) as a colorless oil. The (*S*)-(−)-1-phenylethylamine salt (30.0 mg, 0.055 mmol) was treated with Pd-black (3.0 mg) under a hydrogen atmosphere, followed by purification using Diaion® HP-20SS column chromatography eluted with water and then recrystallization from water–EtOH to give compound **25b** (12.5 mg) in 42% yield as a colorless powder: ^1H NMR (500 MHz, D_2O) δ 4.52 (1H, dd, J = 11.6, 1.7 Hz, H-3), 4.16 (1H, ddd, J = 15.9, 10.5, 5.0 Hz, H-7a), 3.39 (1H, td, J = 11.0, 15.9 Hz, H-7b), 1.96–2.11 (3H, m, H-4a, H-5a, and H-6a), 1.84 (1H, m, H-4b), 1.80 (1H, m, H-5b), 1.50 (1H, m, H-6b); ^{13}C NMR (125 MHz, D_2O) δ 177.8 (C-2), 57.0 (C-3), 48.0 (C-7), 31.2 (C-4), 30.5 (C-6), 29.1 (C-5); ^{31}P NMR (202 MHz, D_2O) δ 6.82; HR-ESIMS *m*/*z* calcd for $C_6H_{14}N_4O_5PS$ $(M-H)^-$ 285.0423, found 285.0414.

7.25. (3*S*)-3-Amino-1-diaminophosphinyl-2-pyrrolidinone (27a)

To a solution of compound **21a** (401.5 mg, 1.29 mmol) in EtOH (14.0 mL) and water (0.8 mL) was added Pd-black (40.0 mg), and the suspension was stirred for 5 h at room temperature under a hydrogen atmosphere. The reaction mixture was filtrated, and the filtrate was evaporated under reduced pressure. The residue was crystallized by trituration in Et_2O to give compound **27a** (132.5 mg) in 54% yield as a colorless powder: ^1H NMR (400 MHz, D_2O) δ 3.79 (1H, dd, J = 11.2, 8.3 Hz, H-3), 3.71 (1H, m, H-5a), 3.58 (1H, td, J = 10.2, 6.4 Hz, H-5b), 2.49 (1H, m, H-4a), 1.88 (1H, m, H-4b); ^{13}C NMR (100 MHz, D_2O) δ 183.2 (C-2), 56.2 (C-3), 46.7 (C-5), 30.7 (C-4); ^{31}P NMR (243 MHz, D_2O) δ 13.86; HR-ESIMS *m*/*z* calcd for $C_4H_{11}N_4NaO_2P$ $(M+Na)^+$ 201.0517, found 201.0520.

7.26. (3*S*)-3-Amino-1-diaminophosphinyl-2-perhydroazepinone 1/2 (+)-dibenzoyl-D-tartarate (27b)

The synthetic method similar to that noted for compound **6** was used. Compound **21b** (501.0 mg, 1.472 mmol) was treated with Pd-black (25.0 mg) under a hydrogen atmosphere. The reaction mixture was filtrated, and addition of (+)-dibenzoyl-D-tartaric acid (277.0 mg, 0.736 mmol) gave compound **27b** (483.0 mg) in 85% yield as a colorless powder: ^1H NMR (400 MHz, D_2O) δ 8.13 (2H, dd, J = 7.3, 1.0 Hz, COPh), 7.71 (1H, t, J = 7.3 Hz, COPh), 7.57 (2H, t, J = 7.3 Hz, COPh), 5.72 (1H, s, OCHCOO), 4.49 (1H, br d, J = 10.7 Hz, H-3), 4.09 (1H, ddd, J = 15.6, 11.2, 5.4 Hz, H-7a), 3.37 (1H, td, J = 11.2, 15.6 Hz, H-7b),

1.90–2.09 (3H, m, H-4a, H-5a, and H-6a), 1.70–1.90 (2H, m, H-4b and H-5b), 1.45 (1H, m, H-6b); ^{13}C NMR (100 MHz, D_2O) δ 177.9 (C-2), 175.9 (CH*CO*O), 170.7 (Ph*CO*O), 136.9 (Ph), 132.7 (Ph × 2), 131.8 (Ph), 131.6 (Ph × 2), 78.1 (O*CH*COO), 57.0 (C-3), 47.5 (C-7), 31.2 (C-4), 30.4 (C-6), 29.1 (C-5); ^{31}P NMR (243 MHz, D_2O) δ 18.30; HR-ESIMS *m/z* calcd for $C_6H_{15}N_4NaO_4P$ $(M+Na)^+$ 229.0830, found 229.0824.

7.27. (3*R*)-3-Amino-1-diaminophosphinyl-2-piperidinone 1/2 (−)-dibenzoyl-L-tartarate (27c)

The synthetic method similar to that noted for compound **6** was used. (3*R*)-3-Benzyloxycarbonylamino-1-diaminophosphinyl-2-piperidinone (**26**) (700.0 mg, 2.145 mmol) was treated with Pd-black (35.0 mg) under a hydrogen atmosphere. The reaction mixture was filtrated, and addition of (−)-dibenzoyl-L-tartaric acid (403.7 mg, 1.073 mmol) afforded compound **27b** (659.8 mg) in 83% yield as a colorless powder: ^{1}H NMR (400 MHz, D_2O) δ 8.13 (2H, d, J = 7.8 Hz, COPh), 7.72 (1H, m, COPh), 7.57 (2H, t, J = 7.8 Hz, COPh), 5.72 (1H, s, OCHCOO), 4.10 (1H, dd, J = 11.7, 6.8 Hz, H-3), 3.65 (2H, td, J = 5.9, 4.9 Hz, H-6), 2.37 (1H, m, H-4a), 1.79–2.07 (3H, m, H-4b and H-5); ^{13}C NMR (100 MHz, D_2O) δ 175.9 (CH*CO*O), 174.6 (C-2), 170.7 (Ph*CO*O), 136.9 (Ph), 132.7 (Ph × 2), 131.8 (Ph), 131.6 (Ph × 2), 78.1 (O*CH*COO), 53.3 (C-3), 47.3 (C-6), 26.6 (C-4), 23.1 (C-5); ^{31}P NMR (243 MHz, D_2O) δ 17.33; HR-ESIMS *m/z* calcd for $C_5H_{13}N_4NaO_2P$ $(M+Na)^+$ 215.0674, found 215.0683.

7.28. In vitro assay for DPP-IV inhibitory activity

0.1 M Tris(hydroxymethyl)-aminomethane/maleic acid buffer solution (pH 7.2, 100 μL), 3.2 mM Gly-Pro-β-naphthylamide (25 μL, Bachem, Switzerland), and an aqueous solution (50 μL) of the synthetic compound were added into 96 well micro-plates. The resultant solution was warmed for 10 min at 37 °C, and then the DPP-IV solution (25 μL) was added to the solution. The combined solution was allowed to react for 1 h at 37 °C. The reaction was terminated by adding a solution (100 μL) of 0.2% Fast Garnet GBC salt (Sigma, USA) in 0.5 M sodium citrate buffer solution (pH 3.78) including 10% polyoxyethylene (20) sorbitan monolaulate (Wako, Japan). The absorbance at 525 nm was measured (value *a*). Simultaneously, the absorbance of the reaction mixture without the synthetic compound solution was measured (value *b*). Moreover, the absorbance of the reaction mixture without the DPP-IV solution was measured, respectively (value a' and value b'). The DPP-IV inhibitory rate (%) was calculated by $[(b-b')-(a-a')/(b-b')]\times 100$. From the inhibitory curve, taking inhibitory rate (%) at various concentrations of the synthetic compound on the ordinate and the logarithm of concentrations of the synthetic compound on the abscissa, the concentration for 50% inhibition was obtained. In all cases, a linear relation was observed between 20% and 80% inhibition. This procedure was repeated three times or more, and each average was shown as the IC_{50} value of the synthetic compound.

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References and notes

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