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Development of a Large-Scale Synthesis of Sulphostin, a Dipeptidyl Peptidase IV Inhibitor

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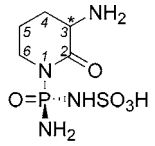
Abstract:

For the progress of the in vivo study on sulphostin, a dipeptidyl peptidase IV inhibitor, its large-scale synthetic method was investigated. The optical resolution of (3*S*,*RS*$_P$)-1-amino(sulfoamino)phosphinyl-3-benzyloxycarbonylamino-2-piperidinone, which was the most difficult step in the previous method, was simplified by using fractional crystallization. The use of 2 mol equiv of (1*S*,2*R*)-(+)-2-amino-1,2-diphenylethanol for optical resolution gave desired diastereomer 15 in good yield as a less soluble salt. In the present synthetic method, there were no requirements for purification using column chromatography, reaction at cryogenic temperature, and treatment using the haloalkane solvents. The total yield of the new method was 4.6%, which was an improvement of approximately 2-fold compared to the method reported previously.

Introduction

Sulphostin (**1**), a dipeptidyl peptidase IV (DPP-IV, CD26, EC 3.4.14.5) inhibitor (IC_{50} = 21 nM), which has two asymmetric atoms at C-3 and phosphorus, was isolated from the culture broth of *Streptomyces* sp. MK251-43F3.[1] We synthesized four possible stereoisomers of sulphostin and determined the configurations of the natural product by comparison with the four stereoisomers.[2] Results of the synthetic study revealed that 3-epi sulphostin (**2**) also had a strong inhibitory activity (IC_{50} = 31 nM) (Figure 1).

DPP-IV is a cell surface serine exopeptidase, which selectively cleaves the N-terminal dipeptides from polypeptides with proline or alanine residue at the penultimate position.[3−5] Glucagon-like peptide-1 (7−36 amide, GLP-1),



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

Figure 1.

an insulin-releasing hormone, is cleaved and inactivated by DPP-IV,[6] and DPP-IV inhibitors suppress the rise in glucose level in blood after a meal.[7,8] The potent DPP-IV inhibitors such as NVP-DPP728[9] and P32/98[10] are currently being evaluated in clinical trials as therapeutic agents for 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.[11,12] It has been confirmed that two DPP-IV inhibitors, Ala-boroPro[13] and prodipine,[14] suppress the immune response in vivo. The DPP-IV inhibitor has been expected to be a therapeutic agent for type 2 diabetes or immune-related disorders. Thus, sufficient quantities of sulphostin were a requisite for the in vivo studies.

However, in our previously used method,[2] three difficult steps were involved in large-scale synthesis; i.e., separation of the diastereomeric mixture using column chromatography, reaction at a cryogenic temperature, and purification of the reaction product using haloalkane solvents. Hence, to circumvent these difficult steps, the development of a new synthetic method was required. We evaluated the improvement in yield in the available synthetic method of sulphostin and simplification of reactions for scale-up. In this paper, we report the large-scale synthesis of sulphostin including

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Scheme 1

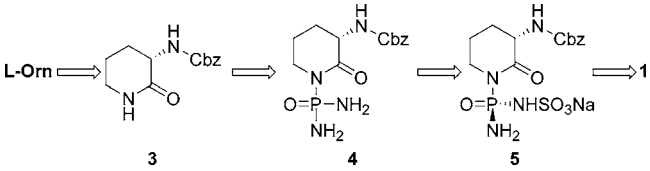
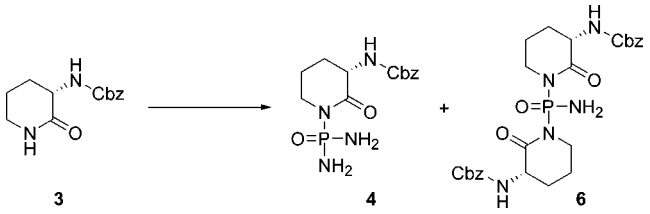


Table 1. **Various reagents for activation of lactam amide (3)**



run	reagents	solvents	$POCl_3$	temp	results[a]
1	$NaHCO_3$	THF	1 equiv	rt	no reaction
2	$^iPr_2EtN$	THF	1 equiv	rt	no reaction
3	$^iPr_2EtN$	toluene	1 equiv	rt	no reaction
4	NaH	THF	1 equiv	−78°C−rt	multiple products
5	TMS−Cl, $^iPr_2EtN$	THF	1 equiv	rt	**3** + **4** + **6**
6	TMS−Cl, $^iPr_2EtN$	toluene	1 equiv	rt	**3** + **4** + **6**
7	TMS−Cl, $^iPr_2EtN$	toluene	4 equiv	rt	**3** + **4**

[a] The results were determined by analyzing the spots on TLC after compound **3** was treated with an activating reagent and phosphoryl chloride, followed by bubbling with gaseous ammonia.

the practical separation method of the diastereomeric mixture by fractional crystallization.[15]

Results and Discussion

The previously used synthetic route of sulphostin is shown in Scheme 1.[2] Since this synthetic route was simple and short, we decided to modify it for large-scale synthesis. (3*S*)-3-Benzyloxycarbonylamino-2-piperidinone (**3**) could be easily prepared from L-ornithine (L-Orn) hydrochloride by using the previous method.[2] Therefore, we focused on the difficult steps: diaminophosphinylation of compound **3**, sulfonation of (3*S*)-3-benzyloxycarbonylamino-1-diaminophosphinyl-2-piperidinone (**4**), and separation of the diastereomeric mixture.

Diaminophosphinylation of (3*S*)-3-Benzyloxycarbonylamino-2-piperidinone (3). In the previous report,[2] lactam amide of compound **3** was activated with *n*-butyllithium at −78 °C, and liquid ammonia was added to the reaction mixture for amidation of the dichlorophosphinyl intermediate. Since this reaction was carried out at a cryogenic temperature, special facilities were required and also attention was paid to use a volatile amidation reagent. Moreover, the reaction product was extracted with $CHCl_3$−MeOH (19:1) for purification. These steps were not an option in the case of large-scale synthesis.

First, we examined the activating reagent of lactam amide to determine if it can be safely used at room temperature (Table 1); treatment with sodium hydrogen carbonate and diisopropylethylamine did not give desired compound **4**. In the case of sodium hydride, although the reaction proceeded, a number of spots were observed on TLC. Next, the activation[16] by the treatment with chlorotrimethylsilane and diisopropylethylamine in THF at room temperature was examined. Diaminophosphinylation by silylation was successful and resulted in the formation of desired compound **4** and bis((3*S*)-3-benzyloxycarbonylamino-2-piperidinone-1-yl)phosphinylamide (**6**) along with the starting material **3**. Diaminophosphinylation in toluene also gave a similar result. Next, the amount of phosphoryl chloride was examined, and the employment of 4 equiv revealed that only compound **4** was produced.

Table 2. **Stability of compound 4 in various pH conditions for 1 h**

run	pH	temp	**4**	**3**
1	6.0	rt	97.7	<1.0
2	8.0	rt	>99	<1.0
3	9.0	rt	97.5	2.5
4	10.0	rt	85.1	12.5
5	10.6	0 °C	82.7	4.8
6	11.0	rt	26.8	71.7
7	11.0	0 °C	67.3	31.7
8	13.7	0 °C	62.7	37.3

Then, amidation using an aqueous ammonia solution instead of a gaseous reagent was attempted, and a similar result was obtained. However, it was proven that compound **4** decomposed into compound **3** in the basic solution. Thus, we investigated the stability of compound **4** in the basic solution in order to determine the pH of the aqueous ammonia solution (Table 2). The results showed that compound **4** decomposed into compound **3** in the solution with a pH value higher than 11 even at 0 °C, and it was comparatively stable in the solution between pH 7 and near pH 10. On the basis of these findings, the dichlorophosphinyl intermediate was treated with aqueous ammonia solution with the pH value of 10.6 in an ice bath; this solution was prepared with 14% aqueous ammonia saturated with ammonium chloride. However, the pH value of the aqueous ammonia solution becomes acidic since hydrogen chloride is produced from the dichlorophosphinyl intermediate and excess phosphoryl chloride when the reaction mixture is added into the ammonia solution. Therefore, the pH value of the ammonia solution was controlled and maintained between 9 and 11 by adding 28% aqueous ammonia solution when the pH value turned under 9.

Next, the purification procedure of compound **4** was examined. Compound **4** and ammonium chloride exist as a precipitate in the above-mentioned reaction mixture, and compound **4** scarcely dissolved in the nonpolar organic solvents with the exception of haloalkane solvents. Thus, we compared the solubilities of compound **4** and ammonium chloride in several solvents to examine the purification technique not by the liquid−liquid extraction but by the solid−liquid extraction. The solubility of compound **4** in THF−MeOH (1:1) was approximately 10-fold higher than

(15) The corresponding patent: WO2003051895/US20050020834.

(16) Murakami, M.; Sato, N.; Hashimoto, S.; Kawamura, T. JP Patent 5,4012,-391, 1979.

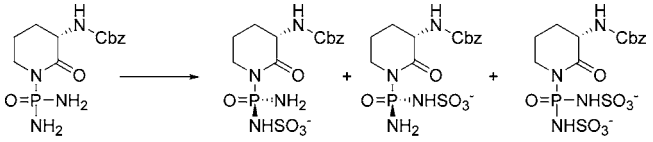
that of ammonium chloride. In contrast, the solubility of compound **4** in water was 2 mg/mL or lower, and that of ammonium chloride was high. In the case of ^{i}PrOH, $^{i}Pr_2O$−EtOH (5:1), and some aprotic solvents, the solubility of compound **4** was 2 mg/mL or lower. From these results, toluene, a reaction solvent, and toluene−THF (1:1) were chosen for the removal of some lipophilic materials from the precipitate containing ammonium chloride and compound **4**, and then THF−MeOH (1:1) was selected for the extraction of compound **4** from the residual precipitate. Moreover, water was selected for the removal of ammonium chloride from the solid residue, which was obtained by evaporation of the solution extracted with THF−MeOH (1:1), and then $^{i}Pr_2O$−EtOH (5:1) was chosen for the removal of the trace lipophilic materials and water.

Consequently, the diaminophosphinylation process was carried out in seven continuous procedures: treatment of compound **3** with chlorotrimethylsilane and diisopropylethylamine, addition of phosphoryl chloride, pouring of the reaction mixture into the aqueous ammonia solution with pH 9−11, collection of the precipitate in the aqueous solution by filtration, washing of the precipitate with toluene and toluene−THF (1:1), extraction of compound **4** with THF−MeOH (1:1), followed by evaporation and washing of the resultant solid residue with water and then a mixture of $^{i}Pr_2O$−EtOH (5:1). The desired compound **4** was obtained by these procedures in 41% yield (purity 98%, optical purity >99% de).

Sulfonation of (3*S*)-3-Benzyloxycarbonylamino-1-diaminophosphinyl-2-piperidinone (4). In the previous report,[2] sulfonation of compound **4** using sulfur trioxide gave the desired compounds in low yield. On investigating the byproduct, it was found that the cause of the low yield was the production of (3*S*)-3-benzyloxycarbonylamino-1-bis(sulfoamino)phosphinyl-2-piperidinone (**9**) due to the overreaction. Thus, reaction temperature, the reagent, and its quantity were carefully examined to suppress the production of compound **9** (Table 3). The reactivity changed when a different complex of sulfur trioxide was used (DMF > pyridine > Me_3N). However, the production of compound **9** could not be suppressed, despite reexamination of the reaction temperature, the quantity of the reagent, and its complex. Pyridine/sulfur trioxide complex (1.2 equiv), which gave the desired compound **8** in best yield, was chosen for sulfonation.

Separation of (3*S*,*RS*$_P$)-1-Amino(sulfoamino)phosphinyl-3-benzyloxycarbonylamino-2-piperidinone (10). In the previous report,[2] separation of the diastereomeric mixture was performed by repeated Diaion HP-20SS column chromatography. In the case of the large-scale synthesis, purification using column chromatography is not practical. Hence, separation of the diastereomeric mixture by fractional crystallization was examined. We hypothesized that the diastereomeric mixture could be separated by preparing the salt with an optically active amine. The various optically active amines[17] for fractional crystallization were examined, and it was found that addition of 1 equiv of (1*S*,2*R*)-(+)-2-amino-1,2-diphenylethanol (ADPE) selectively gave compound **11**, as a less soluble salt, in which the configuration at the phosphorus atom was *S* (Scheme 2). The optical purity of compound **11** was 90% de, which was determined by HPLC analysis. Additionally, the desired diastereomer **12**, in which the configuration of the phosphorus atom was *R*, in the mother liquid had similar optical purity of 90% de. Deprotection of compound **11** gave P-epi sulphostin (**13**) with the optical purity of 98.5% de.

Table 3. **Sulfonation of compound 4 using various reagents for 1 h**



4 **7** **8** **9**

run	reagents	temp	additional reagents	results[a] **4** (%)	**7** (%)	**8** (%)	**9** (%)
1	SO_3·py (1.2 equiv)	4 °C		25.1	31.4	31.9	11.7
2	SO_3·py (2.0 equiv)	4 °C		1.5	24.7	22.5	50.8
3	SO_3·py (1.2 equiv)	50 °C	py (1.2 equiv)	15.6	28.2	29.5	21.0
4	SO_3·DMF (1.2 equiv)	4 °C		11.4	28.2	28.2	17.1
5	SO_3·DMF (1.2 equiv)	4 °C	lutidine (1.2 equiv)	27.7	29.5	28.2	13.8
6	SO_3·Me_3N (1.2 equiv)	4 °C		95.5	0	0	0
7	SO_3·Me_3N (1.2 equiv)	rt, 24 h		79.7	3.2	3.2	0.2

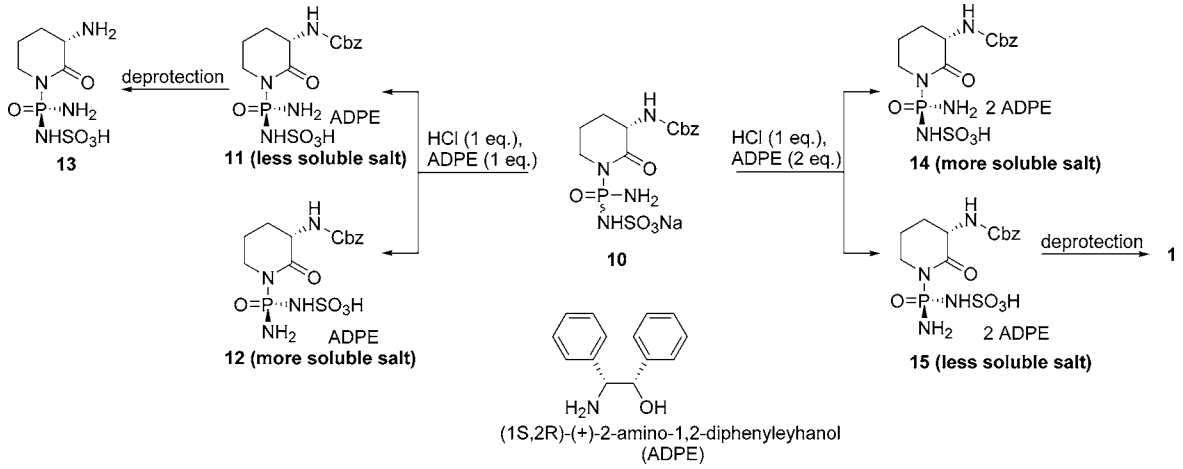
[a] The results were determined by reversed-phase HPLC analysis. Compound **3** was also detected as a decomposition product of compound **4**.

On the other hand, the addition of 2 equiv of ADPE selectively gave compound **15** having the desired configuration at the phosphorus atom as a less soluble salt. The optical purity of compound **15** was 95% de. Therefore, we selected the method using 2 equiv of ADPE for the separation of the diastereomeric mixture. After deprotection of compound **15** by hydrogenolysis in the aqueous acetic acid solution, the desired compound was precipitated by adding EtOH. In this reaction, ADPE was easily removed because an acidic solution was used. Next, recrystallization of resultant crude sulphostin from water−EtOH yielded the pure material, of which the optical purity was >99% de. Consequently, it was proven that the addition of 2 equiv of ADPE resulted in the desired diastereomer **15** with a high optical purity in good yield, and pure sulphostin (**1**) was obtained from compound **15**.

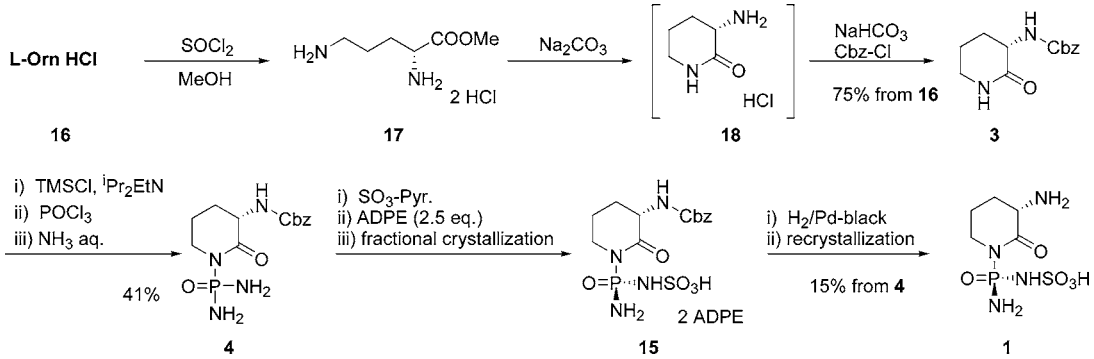
Large-Scale Synthesis of Sulphostin. On the basis of these results, the large-scale synthesis of sulphostin was carried out by the method shown in Scheme 3. L-Orn

(17) Ten or over kinds of optically active amines (e.g., *S*-(−)-1-phenylethylamine, *R*-(+)-1-phenylethylamine, *R*-(−)-2-amino-1-butanol, *R*-(+)-1-(1-naphthyl)-ethylamine, etc.) were examined; however, crystallization of resultant sulfonic acid salts of those amines could not be performed. In the case of (1*R*,2*S*)-(−)-2-amino-1,2-diphenylethanol, the resultant salt was crystalline, which showed no optical activity.

Scheme 2



Scheme 3



hydrochloride (**16**) (1250 g) in MeOH was treated with thionyl chloride at 55 °C to give L-Orn-OMe dihydrochloride (**17**). Cyclization of compound **17** with the aqueous sodium carbonate solution was carried out at room temperature. Continuous addition of sodium hydrogen carbonate and benzyl chloroformate into the cyclization mixture gave compound **3** (purity >99%, optical purity >99% ee) in 75% yield from compound **16**. Reaction of compound **3** (600 g) with chlorotrimethylsilane and diisopropylethylamine at room temperature, followed by treatment with phosphoryl chloride, gave a phosphinyl chloride intermediate, which was poured into an aqueous ammonia solution with the pH value of 9−11 to give compound **4** (purity >99%, optical purity >99% ee) in 44% yield. After treatment of compound **4** (294 g) with 1.2 equiv of pyridine/sulfur trioxide complex at 0−5 °C, addition of 2.5 equiv of ADPE to the reaction mixture resulted in sulfonic acid 2 ADPE salt (**15**) (optical purity 92% de). Deprotection of compound **15** was carried out by hydrogenolysis using palladium-black catalyst in the aqueous acetic acid solution to give crude sulphostin (purity 98.5%, optical purity 95% de) in 20% yield from compound **4**. The crude material was recrystallized from water−EtOH to give pure sulphostin (**1**) (purity >99%, optical purity >99% de) in 74% yield. The physicochemical and biological properties of the pure material were identical with those of previously reported sulphostin.[2] Finally, we examined the recovery of ADPE because it is an expensive reagent and 76% of ADPE was recovered from the optical resolution and deprotection procedure.

Conclusion

The previously reported method for synthesis of sulphostin was reexamined in order to improve its large-scale synthesis. The optimized procedure does not require cryogenic temperature reaction, column chromatography, and the use of haloalkane solvents. The yield of sulphostin from compound **3** was 6.1%, which was a 2-fold improvement in comparison with that of our previously reported method.[2] The required time for the synthesis of sulphostin had been drastically reduced because of the discovery of fractional crystallization using 2 equiv of ADPE. Additionally, the large-scale synthesis of 3-epi sulphostin (**2**), which also had a strong DPP-IV inhibitory activity, from D-Orn hydrochloride could be possible using a similar method, which employed 1 equiv of (1*R*,2*S*)-(−)-2-amino-1,2-diphenylethanol as an optically active amine reagent for the separation of the diastereomeric mixture.

Experimental Section

General Experimental Procedures. Optical rotations were measured with a Perkin-Elmer Model 241 polar meter. ^{1}H NMR, ^{13}C NMR, and ^{31}P NMR spectra were recorded with a Varian Gemini 200, Bruker Avance 400, or 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 spectra. HR-ESIMS spectra were measured with a JEOL JMS-T100LC.

(3*S*,*SR*$_P$)-1-Amino(sulfoamino)phosphinyl-3-benzyloxycarbonylamino-2-piperidinone Sodium Salt (10). To a solution of compound **4** (10.00 g, 30.6 mmol) in DMF (80 mL) was added SO_3/pyridine complex (6.35 g, 40.0 mmol) at 0 °C, and the mixture was stirred for 12 h at 6−8 °C. To the mixture were added water (100 mL) and $NaHCO_3$ (6.72 g, 80.0 mmol), and the mixture was stirred for 5 min at room temperature. The reaction mixture was diluted with water (400 mL) and was purified by Diaion HP-20SS column chromatography (water−30% MeOH(aq)) to give compound **10** (5.80 g) in 45% yield. ^{1}H NMR (D_2O, 600 MHz) δ: 1.73−2.07 (3H, m, H-4a, H-5), 2.21 (1H, m, H-4b), 3.56−3.82 (2H, m, H-6), 4.23 and 4.31 (1H, m, H-3), 5.13−5.23 (2H, m, O*CH*$_2$Ph), 7.42−7.50 (5H, m, Ph); ^{31}P NMR (D_2O, 243 MHz) δ: 4.87 and 5.31.

Separation of (3*S*,*RS*$_P$)-1-Amino(sulfoamino)phosphinyl-3-benzyloxycarbonylamino-2-piperidinone Sodium Salt (10) Using 1 equiv of (1*S*,2*R*)-(+)-2-Amino-1,2-diphenylethanol (ADPE). To a solution of compound **10** (1.05 g, 2.45 mmol) in water (50 mL) were added ADPE (0.522 g, 2.45 mmol) and 1 N HCl(aq) (2.45 mL, 2.45 mmol). The suspension was dissolved by heating to 90 °C and was allowed to cool to room temperature. The precipitate was collected by filtration to give (3*S*,*S*$_P$)-1-amino(sulfoamino)-phosphinyl-3-benzyloxycarbonylamino-2-piperidinone ADPE salt (**11**) (0.66 g) in 42% yield. The optical purity of compound **11** was 90% de, and that of (3*S*,*R*$_P$)-compound **12** in the filtrate was also 90% de. Each optical purity was measured by reversed-phase HPLC (conditions; column, Senshu Pak PEGASIL ODS 4.6 mm × 250 mm (rt); eluent, MeCN/1% phosphoric acid(aq) = 14:86; flow rate, 1.0 mL/min; detection, UV 210 nm). The retention times of compounds **11**, **12**, and ADPE were 27, 29, and 17 min, respectively.

Physicochemical Data of Compound 11: ^{1}H NMR (CD_3OD, 600 MHz)[18] δ: 1.77 (1H, m, H-4a), 1.85−2.00 (2H, m, H-5), 2.21 (1H, m, H-4b), 3.63 (1H, m, H-6a), 3.79 (1H, m, H-6b), 4.23 (1H, m, H-3), 4.41 (1H, d, J = 4.8 Hz, *CH*(NH_2)CH(OH)), 5.07 (2H, s, O*CH*$_2$Ph), 5.16 (1H, d, J = 4.8 Hz, CH(NH_2)*CH*(OH)), 7.09 (2H, m, Ph), 7.17−7.22 (6H, m, Ph), 7.23−7.30 (4H, m, Ph), 7.31−7.36 (3H, m, Ph); ^{13}C NMR (CD_3OD, 150 MHz)[18] δ: 22.4 (C-5), 28.1 (C-4), 45.9 (C-6), 53.4 (C-3), 61.8 ($CHNH_2$), 67.6 (O*CH*$_2$-Ph), 75.1 (CHOH), 127.6 (Ph), 128.8 (Ph), 128.9 (Ph), 129.0 (Ph), 129.1 (Ph), 129.2 (Ph), 129.6 (Ph), 129.8 (Ph), 138.2 (Ph), 142.0 (Ph), 158.5 (NHCOO), 175.7 (C-2); ^{31}P NMR (CD_3OD, 243 MHz) δ: 6.04; HR-ESIMS *m*/*z*: calcd for $C_{13}H_{18}N_4O_7PS$ (M−ADPE−H)$^-$ 405.0634, found 405.0627.

(3*S*,*S*$_P$)-3-Amino-1-amino(sulfoamino)phosphinyl-2-piperidinone: P-epi Sulphostin (13). To a suspension of compound **11** (73.56 g, 116 mmol, 88.4% de) in acetic acid (150 mL) and water (375 mL) was added Pd-black (3.65 g), and the suspension was stirred for 24 h at room temperature under a hydrogen atmosphere. The reaction mixture was filtrated to remove Pd-black, and the residue was washed with water (500 mL). The filtrate and the washing solution were combined, and EtOH (2.0 L) was slowly added to the combined aqueous solution. The precipitate was collected by filtration to give compound **13** (24.5 g, 98.5% de) in 71% yield. To prepare the optical analytical specimen, compound **13** (20 mg) was reacted with benzyl chloroformate (0.025 mL) and $NaHCO_3$ (33 mg) in water (10 mL) and THF (5 mL) at room temperature. After 30 min, the aqueous solution of the reaction mixture was measured by reversed-phase HPLC analysis. The optical purity was obtained from a rate of diastereomer in this reaction mixture. $[\alpha]_D^{20}$ +43.8° (*c* 0.5, H_2O); ^{1}H NMR (400 MHz, D_2O) δ: 1.85−2.12 (3H, m, H-4a and H-5), 2.41 (1H, m, H-4b), 3.63−3.74 (2H, m, H-6), 4.13 (1H, dd, J = 6.5,7.0 Hz, H-3); ^{13}C NMR (D_2O, 100 MHz) δ: 23.1 (d, C-5), 26.7 (C-4), 47.8 (C-6), 53.4 (d, C-3), 174.5 (C-2).

Separation of (3*S*,*RS*$_P$)-1-Amino(sulfoamino)phosphinyl-3-benzyloxycarbonylamino-2-piperidinone Sodium Salt (10) Using 2 equiv of ADPE. To a solution of compound **10** (6.94 g, 16.2 mmol) in water (200 mL) and EtOH (350 mL) were added ADPE (7.96 g, 37.3 mmol) and 1 N HCl(aq) (16.2 mL, 16.2 mmol). The suspension was dissolved by heating to internal temperature at 55 °C and was allowed to cool to room temperature. The precipitate was collected by filtration to give (3*S*,*R*$_P$)-1-amino(sulfoamino)phosphinyl-3-benzyloxycarbonylamino-2-piperidinone 2 ADPE salt (**15**) (5.40 g) in 40% yield. The optical purity of compound **15** was 95% de, and that of (3*S*,*S*$_P$)-compound **14** in the filtrate was 80% de.

Physicochemical Data of Compound 15: ^{1}H NMR (CD_3OD, 600 MHz)[18] δ: 1.74−1.84 (2H, m, H-4a and H-5a), 2.07 (1H, m, H-5b), 2.14 (1H, m, H-4b), 3.57 (1H, m, H-6a), 3.86 (1H, m, H-6b), 4.27 (1H, m, H-3), 4.28 (2H, d, J = 4.8 Hz, *CH*(NH_2)CH(OH) × 2), 5.04 (2H, d, J = 4.8 Hz, CH-(NH_2)*CH*(OH) × 2), 5.09 (2H, s, O*CH*$_2$Ph), 7.12−7.37 (25H, m, Ph × 5); ^{13}C NMR (CD_3OD, 150 MHz)[18] δ: 21.9 (C-5), 27.7 (C-4), 45.6 (C-6), 53.4 (C-3), 62.1 ($CHNH_2$), 67.7 (O*CH*$_2$Ph), 76.6 (CHOH), 127.8 (Ph), 128.7 (Ph), 128.8 (Ph), 128.9 (Ph), 129.0 (Ph), 129.1 (Ph), 129.4 (Ph), 129.5 (Ph), 138.2 (Ph), 142.0 (Ph), 158.8 (NHCOO), 175.4 (C-2); ^{31}P NMR (CD_3OD, 243 MHz) δ: 5.31; HR-ESIMS *m*/*z*: calcd for $C_{13}H_{18}N_4O_7PS$ (M−2ADPE−H)$^-$ 405.0634, found 405.0644.

Small-scale Synthesis of (3*S*,*R*$_P$)-3-Amino-1-amino-(sulfoamino)phosphinyl-2-piperidinone: Sulphostin (1). To a suspension of compound **15** (1.00 g, 1.20 mmol, 95% de) in acetic acid (2 mL) and water (5 mL) was added Pd-black (0.050 g), and the suspension was stirred for 2 h at room temperature under a hydrogen atmosphere. The reaction mixture was filtrated to remove Pd-black, and the residue was washed with water (6 mL). The filtrate and the washing solution were combined, and then EtOH (21 mL) was slowly added. The precipitate was collected by filtration to give crude sulphostin (0.28 g, 98.6% de) in 81% yield. Recrystallization of crude sulphostin from water−EtOH gave pure compound **1** (>99% de): $[\alpha]_D^{20}$ −21.8° (*c* 5.0, H_2O); ^{1}H NMR (400 MHz, D_2O) δ: 1.85−2.02 (2H, m, H-4a and H-5a), 2.11 (1H, m, H-5b), 2.40 (1H, m, H-4b), 3.65 (1H, m, H-6a), 3.79 (1H, m, H-6b), 4.15 (1H, dd, J = 11.9, 6.9

(18) As an internal standard substance for ^{1}H and ^{13}C NMR spectra, a solvent signal (3.30 and 49.0 ppm) was used, respectively.

Hz, H-3); ^{13}C NMR (100 MHz, D_2O) δ: 22.6 (d, C-5), 26.3 (C-4), 47.5 (C-6), 53.4 (d, C-3), 174.5 (C-2).

Large-Scale Synthesis of Sulphostin. (3*S*)-3-Benzyloxycarbonylamino-2-piperidinone (3). To a suspension of L-ornithine hydrochloride (**16**) (1250 g, 7.40 mol) in MeOH (3.7 L) was slowly added thionyl chloride (568 mL, 7.78 mol) keeping the internal temperature below 0 °C, and the mixture was stirred for 3 h at 50−55 °C. The reaction mixture was evaporated under reduced pressure, and the residue was crystallized by trituration in $^{i}Pr_2O$ (4.0 L). The crystals were collected by filtration and dried at room temperature and then at 40 °C under reduced pressure to give L-ornithine methyl ester dihydrochloride (**17**). The accurate quantity of compound **17** could not be measured due to being hydroscopic. To a solution of compound **17** in water (3.6 L) was slowly added a solution of Na_2CO_3 (1202 g, 11.36 mol) in water (6.0 L) keeping the internal temperature below 5 °C. After the mixture was stirred at the same temperature for 2 h, stirring of the reaction was continued for 10 h at room temperature. To the reaction mixture were added $NaHCO_3$ (954 g, 11.36 mol) and THF (2.1 L). Continuously, benzyl chloroformate (1136 mL, 7.96 mol) was slowly added with keeping the internal temperature below 10 °C. Stirring of the reaction was continued for 1 h at room temperature. The reaction mixture was separated, and the aqueous layer was extracted twice with EtOAc (6.0 and 3.0 L). The organic layers were combined and were washed with 5% citric acid(aq) (4.0 L), 5% $NaHCO_3$(aq) (4.0 L), and saturated NaCl(aq) (4.0 L × 2). The organic solution was dried over anhydrous Na_2SO_4 and was evaporated under reduced pressure until its volume approximately became 3.0 L. To the solution was slowly added $^{i}Pr_2O$ (2.0 L). The crystals were collected by filtration, washed with $^{i}Pr_2O$ (0.5 L), and dried at room temperature and then at 40 °C under reduced pressure to give compound **3** (1383 g, purity >99%, optical purity >99% ee) in 75% yield from L-ornithine hydrochloride (**16**). The optical purity was measured by HPLC analysis using a chiral column (conditions: column, CHIRALPAK AS (4.6 mm × 250 mm); eluent, *n*-hexane/EtOH = 1:1; flow rate, 0.4 mL/min; detection, UV 215 mm). The retention time of compound **3** was 25.4 min. For reference, that of 3-epimer of compound **3** was 47.2 min. ^{1}H NMR ($CDCl_3$, 200 MHz) δ: 1.61 (1H, tdd, J = 11.7, 5.4, 12.7 Hz, H-4a), 1.82−1.95 (2H, m, H-5), 2.53 (1H, m, H-4b), 3.27−3.33 (2H, m, H-6), 4.09 (1H, m, H-3), 5.11 (2H, s, O*CH*$_2$Ph), 5.79 (1H, br s, NHCOO), 6.36 (1H, br s, H-1), 7.28−7.38 (5H, m, Ph).

(3*S*)-3-Benzyloxycarbonylamino-1-diaminophosphinyl-2-piperidinone (4). To a suspension of compound **3** (600 g, 2.52 mol) in toluene (6.0 L) were added chlorotrimethylsilane (612 mL, 4.80 mol) and diisopropylethylamine (823 mL, 4.80 mol), and the mixture was stirred for 24 h at room temperature. To the mixture was added phosphoryl chloride (894 mL, 9.60 mol), and the mixture was stirred for 2 days at room temperature. The reaction mixture was poured into the aqueous ammonia solution prepared from NH_4Cl (1596 g), 28% ammonia(aq) (3420 mL), and water (2850 mL) keeping the pH of the ammonia solution at 9−11 by addition of 28% ammonia(aq) in an ice bath. The precipitate was collected by filtration and was washed with toluene (6.0 L) and a solution (4.5 L) of toluene−THF (1:1), followed by extraction with a solution (10 L) of THF−MeOH (1:1). After evaporation of the extract under reduced pressure, the solid residue was washed with water (6.0 L) and then with a solution (10 L) of EtOH− $^{i}Pr_2O$ (1:5). The precipitate was dried at 40 °C under reduced pressure to give compound **4** (323 g, purity 98.4%, optical purity >99% ee) in 41% yield. The optical purity was measured by HPLC analysis using a chiral column (conditions were similar to those described for compound **3**). The retention time of compound **4** was 18.9 min. For reference, that of 3-epimer of compound **4** was 15.7 min. ^{1}H NMR (DMSO-d_6, 200 MHz) δ: 1.62 (1H, m, H-4a), 1.73−1.78 (2H, m, H-5), 2.01 (1H, m, H-4b), 3.46 (1H, m, H-6a), 3.58 (1H, m, H-6b), 4.09 (1H, m, H-3), 4.14 (2H, br s, PNH_2), 4.19 (2H, br s, PNH_2), 5.00 (2H, s, O*CH*$_2$-Ph), 7.28−7.41 (6H, m, Ph and NHCOO).

(3*S*,*R*$_P$)-1-Amino(sulfoamino)phosphinyl-3-benzyloxycarbonylamino-2-piperidinone 2 ADPE Salt (15). A suspension of compound **4** (294 g, 0.90 mol) in DMF (3.0 L) was dissolved by heating internal temperature at 60 °C and was allowed to cool in an ice bath. To the solution was added SO_3/pyridine complex (172 g, 1.08 mol) keeping internal temperature at 0−5 °C, and the mixture was stirred for 1 h. After addition of water (100 mL), the mixture was allowed to warm to room temperature. To the reaction mixture were added MeOH (1.0 L) and a half of the ADPE (480 g, 2.25 mol) solution in MeOH (4.0 L), and the mixture was heated at 50 °C. The remainder of the ADPE solution was added, and the mixture was stirred for 30 min. The mixture was filtrated to remove the precipitate, and the filtrate was evaporated to remove MeOH under reduced pressure. The residue was diluted with EtOH (15.0 L) and water (6.0 L), and the solution was stirred for 16 h at room temperature and then was stirred for 1 h below 10 °C. The precipitate was collected by filtration and was dried at 40 °C under reduced pressure to give compound **15** (257 g, 92% de).

Crude (3*S*,*R*$_P$)-3-Amino-1-amino(sulfoamino)phosphinyl-2-piperidinone: Crude Sulphostin (1). To a suspension of compound **15** (255 g) in acetic acid (400 mL) and water (2.0 L) was added Pd-black (11 g), and the suspension was stirred for 2 h at room temperature under 10 atm of pressure of hydrogen. The reaction mixture was filtrated to remove Pd-black, and the filtrate was diluted with EtOH (5.0 L). The solution was stirred for 2 h keeping internal temperature below 5 °C. The crystals were collected by filtration, washed with EtOH (0.5 L), and dried at 40 °C under reduced pressure to give crude sulphostin (51.6 g, purity 98.6%, optical purity 95.3% de) in 20% yield from compound **4**. The optical purity was measured by the method described for compound **13**.

Purification of Crude Sulphostin (1). A solution of crude sulphostin (150 g, 0.52 mol, 97.2% de) in water (1.80 L) was filtrated by the membrane filter. The filtrate was diluted with EtOH (0.90 L) keeping the internal temperature at 55−60 °C. The solution was stirred for 16 h at room temperature. The crystals were collected by filtration, washed

with a solution (400 mL) of water−EtOH (1:2), and dried at 40 °C under reduced pressure to give pure sulphostin (111 g, purity >99%, optical purity >99% de) in 74% yield. This synthetic sulphostin was identical with the authentic sample[2] in physicochemical and biochemical properties.

Recovery of ADPE (480 g × 3 lots). The precipitate and the aqueous solution containing ADPE were combined and were evaporated under reduced pressure to remove the organic solvents, followed by addition of 2 equiv of 3 N NaOH(aq) against the amount of ADPE calculated by reversed-phase HPLC analysis. The aqueous solution was stirred overnight, and then the crystals were collected by filtration. The crystals were recrystallized from MeOH−water to recover ADPE (1100 g) in 76% yield.

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