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Asymmetric Total Synthesis and Evaluation of Antitumor Activity of Ophiorrhisine A and Its Derivatives

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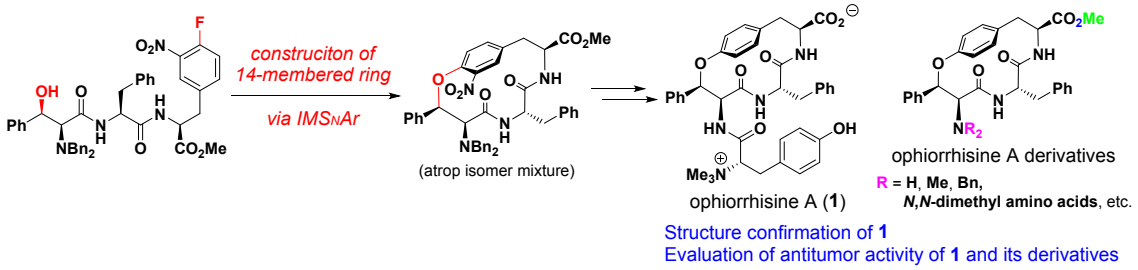


ABSTRACT: The first asymmetric total synthesis of ophiorrhisine A (**1**), a new cyclic tetrapeptide isolated from *Ophiorrhiza nutans*, was accomplished via an intramolecular aromatic nucleophilic substitution reaction (IMSNAr) of a linear tripeptide to construct a 14-membered paracyclophane ring, resulting in confirmation of its structure and absolute configuration. Structure-activity relationship study of **1** and its derivatives demonstrated that some derivatives possessed cytotoxicity toward human cancer cell lines A549, HT29, and HCT116.

INTRODUCTION

Ophiorrhisine A (**1**, Figure 1)[1] is a new cyclic peptide isolated from *Ophiorrhiza nutans* (Rubiaceae) collected in Thailand in the course of our chemical investigation of new and bioactive alkaloids.[2] The structure featuring a 14-membered paracyclophane ring and a novel *N*,*N*,*N*-trimethylated tyrosine residue in the side chain and its absolute configuration were inferred by spectroscopic analysis and biosynthetic considerations. The stereochemistry at C-3, the aryl-alkyl ether bond, was different from that of common known cyclopeptide alkaloids.[3a] Cyclopeptide alkaloids possessing 13-, 14-, or 15-membered cyclophane rings are widely found in several plants belonging to genera Rhamnaceae, Pandaceae, and Rubiaceae.[3a] Owing to their interesting biological properties, such as sedative, antibacterial, antifungal, antitumor, and antiplasmodial activities, total synthetic and/or biological studies of this class of natural products have been pursued.[3] Herein, we report the asymmetric total synthesis of **1** to confirm its structure and absolute configuration, and a structure-activity relationship (SAR) study of **1** and its derivatives, focusing on

antitumor activity.

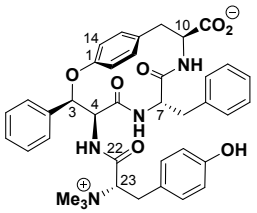
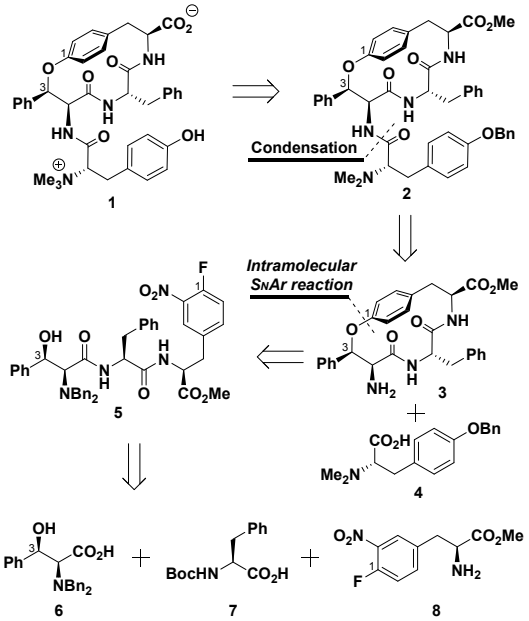


Figure 1. Ophiorrhisine A isolated from *Ophiorrhiza nutans* (Rubiaceae) collected in Thailand.

RESULTS AND DISCUSSION

The retrosynthesis of **1** is shown in Scheme 1. We envisioned that target molecule **1** would be obtained from compound **2** through the hydrolysis of the methyl ester, the methylation of the *N*,*N*-dimethylamine group, and the deprotection of the phenolic hydroxyl group. **2** would be produced by the condensation of cyclic compound **3** and tyrosine derivative **4**. **3** would be constructed through the intramolecular aromatic nucleophilic substitution reaction (IMSNAr) of tripeptide **5**, which would be obtained by the successive condensation of (3*R*)-hydroxyphenylalanine derivative **6** and L-phenylalanine derivatives **7** and **8**.

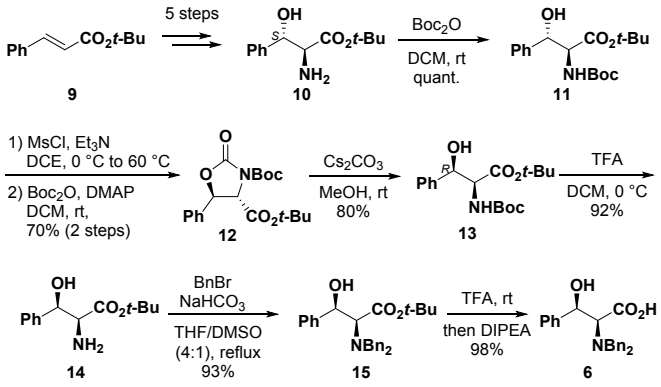
Scheme 1. Retrosynthesis of Ophiorrhisine A (1)



First, we synthesized (3*R*)-hydroxyphenylalanine derivative **6** through the inversion of the stereochemistry of the hydroxyl group in known (3*S*)-hydroxyphenylalanine derivative **10** (Scheme 2). **10** was prepared from cinnamic *tert*-butylate (**9**) according to the Davies method.[4] After Boc protection of the amino group of **10**, **11**[5] was mesylated in dichloroethane at 0 °C. After the reaction was completed as judged by TLC monitoring, the reaction mixture was warmed to 60 °C to induce an intramolecular

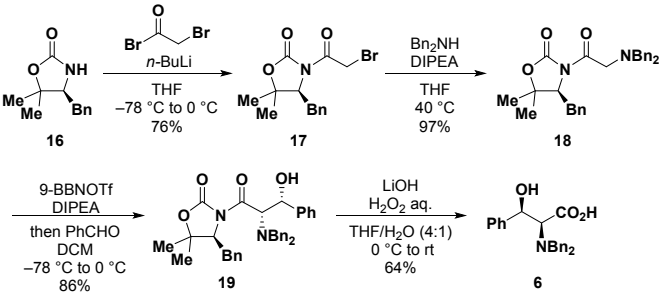
cyclization by the SN2 reaction of the carbonyl group of Boc to give oxazolidinone, which was treated with Boc_2O to afford compound **12**[6] in 70% yield over 2 steps. The oxazolidinone ring of **12** was opened by methanolysis in the presence of Cs_2CO_3 to generate (3*R*)-hydroxyphenylalanine derivative **13**[6] in good yield. Deprotection of the Boc group under acid condition at 0 °C, dibenzylation with BnBr in the presence of $NaHCO_3$, and removal of the *tert*-butyl group with TFA afforded carboxylic acid **6** in excellent yield over 3 steps.

Scheme 2. Synthesis of Carboxylic Acid 6



The synthetic route shown in Scheme 2 provided **6** in good overall yield but required 12 steps to obtain **6** from **9**. Thus, we developed a shorter synthetic route by using the Evans-aldol reaction to directly construct the desired stereochemistry (Scheme 3). Bromoacetylation of (–)-oxazolidinone **16**,[7] which was prepared from L-phenylalanine in 4 steps, afforded known compound **17**.[8] **17** was subjected to amination with dibenzylamine in the presence of DIPEA to give Evans-aldol reaction substrate **18** in good yield. In reference to the Davies method,[8] the Evans-aldol reaction of **18** with 9-BBNOTf, DIPEA, and benzaldehyde afforded oxazolidinone **19** as a single diastereomer. Hydrolysis of **19** under the optimum reaction conditions (H_2O_2, LiOH, THF/H_2O) generated **6** in 64% yield.

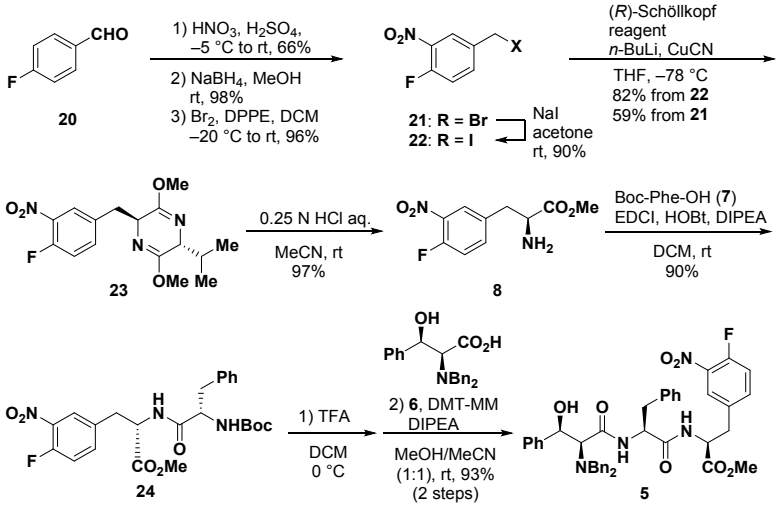
Scheme 3. Alternative Synthetic Route for Carboxylic Acid 6



Next, we synthesized tripeptide **5** from commercially available 4-fluorobenzaldehyde (**20**) (Scheme 4). Nitration of **20**, reduction of aldehyde, and bromination by the Appel reaction yielded bromide **21**,[9]

which was subjected to the Finkelstein reaction to give iodide **22**.[10] Stereoselective alkylation of bromide **21** or iodide **22** with Schöllkopf reagent[11] in the presence of copper cyanide[12] afforded alkylated compound **23** as a single diastereomer. Cleavage of the chiral auxiliary in **23** with dilute aqueous HCl in MeCN gave chiral phenylalanine derivative **8**,[13] which was then coupled with *N*-Boc-L-phenylalanine (**7**) to afford dipeptide **24**. Removal of the Boc group followed by chemoselective condensation with (3*R*)-hydroxyphenylalanine derivative **6** using 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMT-MM)[14] in MeOH/MeCN afforded tripeptide **5** in excellent yield.

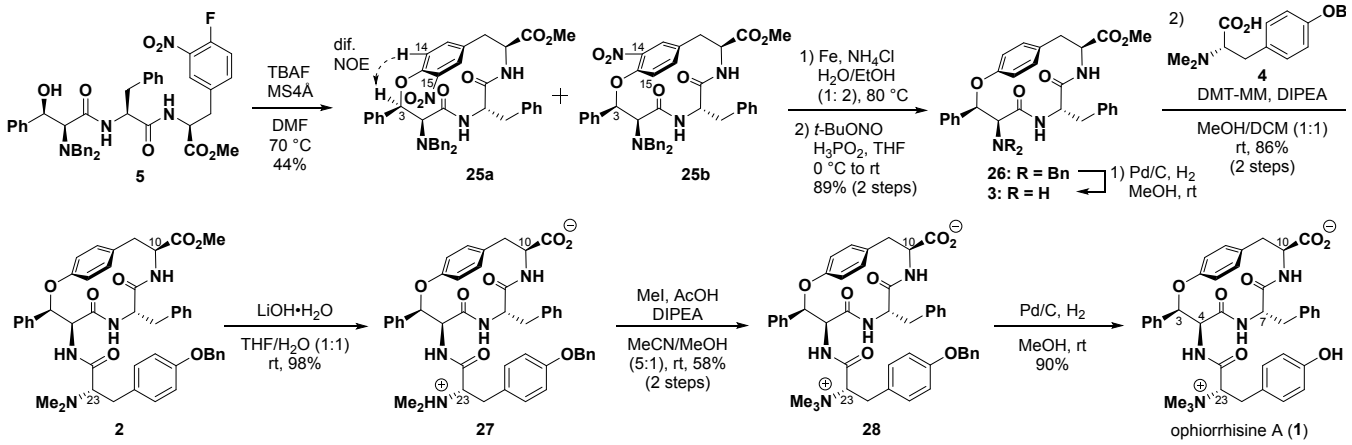
Scheme 4. Synthesis of Tripeptide 5



With tripeptide **5** in hand, the key IMSNAr-based cyclization was performed in reference to the conditions used by Zhu's group[15] (Scheme 5). Under the optimized reaction conditions (TBAF, powdered 4Å molecular sieves, DMF), the cyclization of **5** proceeded to form the 14-membered paracyclophane in 44% yield as a mixture of inseparable atropisomers, **25a** and **25b**, in the ratio of 5:1. The strong NOE correlation between protons H-3 and H-14 in **25a** revealed its configuration, as shown in Scheme 5. Reductive removal of the nitro group in **25a** and **25b** was performed in a two-step operation [1) Fe, NH_4Cl, H_2O/EtOH; 2) *t*-BuONO, H_3PO_2, THF] to provide desired 14-membered cyclopeptide core **26** in 89% yield (2 steps). Hydrogenolysis of **26** in the presence of Pd/C proceeded smoothly to provide primary amine **3**, which was condensed with *N*,*N*-dimethyl-*O*-benzyl-L-tyrosine **4**[16] using DMT-MM in MeOH/DCM to afford cyclophane **2** in 86% yield over 2 steps. After the hydrolysis of **2** with LiOH in THF/H_2O, selective methylation of the dimethylamine in the side chain of **27** with iodomethane in the presence of both acetic acid and DIPEA gave zwitterionic compound **28** possessing a carboxylic acid function and a trimethylated amine function. Finally, removal of the *O*-benzyl group of the tyrosine residue afforded ophiorrhisine A (**1**) in 90% yield. All spectral data of synthetic **1**, including NMR, mass, optical rotation, and experimental circular dichroism (ECD) data, were identical to those of natural

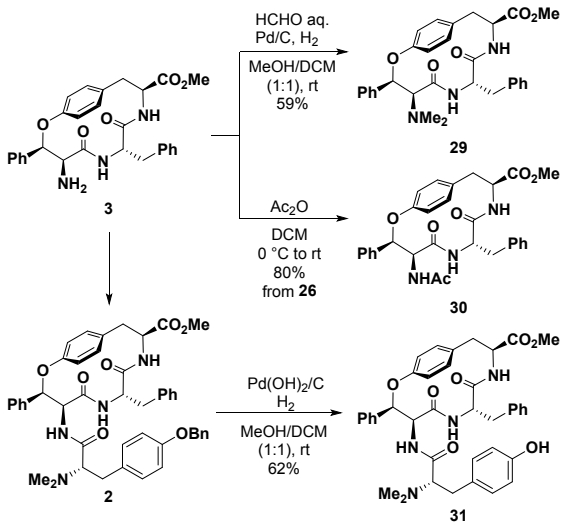
ophiorrhisine A (**1**).[1] Thus, the structure of **1** including its absolute configuration was confirmed.

Scheme 5. Total Synthesis of Ophiorrhisine A (1)

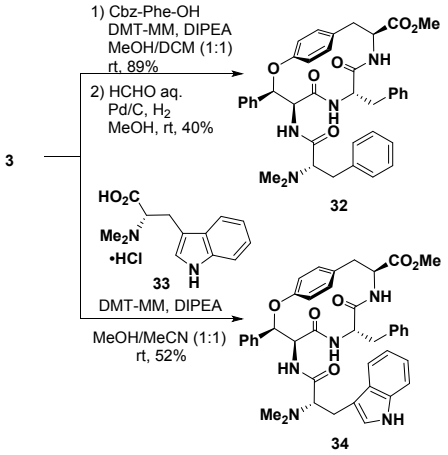


We next set our sights on the syntheses of the derivatives of **1** for SAR studies, focusing on antitumor activity (Schemes 6 and 7). The derivatives were designed by referring to the structures of other natural cyclopeptide alkaloids having significant bioactivities.[3c, 17] The *N*,*N*,*N*-trimethylated tyrosine moiety in **1** was replaced by other residues and the carboxylic acid was capped as methyl ester to reduce polarity and improve cell wall penetration. First, the amino group in **3** was dimethylated with formaldehyde in the presence of Pd/C to give **29**, and was acylated with Ac_2O to afford **30**. Next, we synthesized derivatives possessing *N*,*N*-dimethylated amino acid residues. Removal of the benzyl group of **2** afforded tyrosine derivative **31**. Coupling of **3** with *N*-Cbz-L-phenylalanine followed by reductive amination with formaldehyde gave phenylalanine derivative **32**. The combination of **3** with *N*,*N*-dimethyltryptophan•HCl salt **33** in the presence of DMT-MM and DIPEA afforded tryptophan derivative **34**.

Scheme 6. Syntheses of Ophiorrhisine A Derivatives 29, 30, and 31



Scheme 7. Syntheses of Ophiorrhisine A Derivatives 32 and 34



Synthetic compound **1** and its derivatives **3**, **26**, **29**, **30**, **31**, **32**, and **34** were evaluated for cytotoxicity toward human cell lines A549, HT29, and HCT116 (Table 1). As presumed, compound **1** showed no antitumor activity (IC_{50} > 15 μM). However, derivatives **3**, **26**, **29**, **32**, and **34**, which lack an ionic character compared to **1**, exhibited modest cytotoxicity.

Table 1. Cytotoxicity of Ophiorrhisine A and Derivatives 3, 26, 29, 30, 31, 32, and 34 toward Human Cell Lines A549, HT29, and HCT116

	IC_{50} (μM)		
Sample	A549	HT29	HCT116
Ophiorrhisine A (**1**)	>15	>15	>15
26	>15	7.0	>15
3	>15	>15	11.6
29	10.8	7.1	3.9
30	>15	>15	>15
31	>15	>15	>15
32	9.0	3.5	2.9
34	>15	10.0	8.4
Colchicine	0.0093	0.0075	0.0075

CONCLUSIONS

We have accomplished the first asymmetric total synthesis of ophiorrhisine A (**1**), which enabled us to confirm the inferred structure and the absolute configuration of **1**. Zhu's IMSNAr method to construct a 14-membered paracyclophane ring was applied as the key step. Furthermore, we synthesized derivatives of **1** and investigated the cytotoxicity of **1** and its derivatives toward human cancer cell lines A549, HT29, and HCT116. Ophiorrhisine A derivatives **3**, **26**, **29**, **32**, and **34** showed not significant but modest cytotoxic activity.

EXPERIMENTAL SECTION

General Experimental Procedures. UV: recorded in MeOH on a JASCO V-560 instrument. IR: recorded on a JASCO FT/IR-230 spectrophotometer. ^{1}H and $^{13}C\{^{1}H\}$ NMR spectra: recorded on JNM ECZ-400 and JNM ECS-400 at 400 MHz (^{1}H) or 100 MHz (^{13}C), and JNM ECZ-600 and JNM ECA-600 at 600 MHz (^{1}H) or 150 MHz (^{13}C), respectively. J values are given in Hz. ESIMS: JEOL JMS T100GCV. HRESIMS: JEOL JMS-T100LP AccuTOF LC-plus. ECD: JASCO J-720WI. TLC: precoated silica gel 60 F_{254} plates (0.25 mm thick) and precoated RP-18 F_{254} plates (Merck, Tokyo, Japan), precoated amino-silica gel plates (Fuji Silysia Chemical. Tokyo, Japan). Column chromatography: silica gel 60 (70–230 mesh, Merck, Tokyo, Japan), silica gel 60N [40–50 μm (for flash chromatography), Kanto Chemical, Tokyo, Japan], Chromatorex NH (100–200 mesh, Fuji Silysia Chemical, Tokyo, Japan), Cosmosil 75C_{18}-OPN (Nacalai Tesque, Kyoto, Japan). Medium pressure liquid chromatography (MPLC): C.I.G. prepacked column CPS-HS-221-05 (silica gel, Kusano Kagakukikai, Japan), Ultra Pack NH-40A (amino-silica gel, Yamazen, Osaka, Japan), and Ultra Pack ODS-A-40A (ODS, Yamazen, Osaka, Japan).

t-Butyl Ester ***9***. To a solution of cinnamic acid (2.96 g, 20.0 mmol) in DCM (30 mL, 0.67 M) were added oxalyl chloride (1.8 mL, 1.05 eq.) and DMF (75 μL, 5 mol%) at room temperature under Ar atmosphere. After the reaction mixture was stirred for 1.5 hours at the same temperature, the solvent was removed by evaporation. The residue was dissolved in THF (20 mL) and the solution was cooled to 0 °C. To the solution was added 1 M KO*t*-Bu solution in THF (24 mL, 1.2 eq.) at 0 °C under Ar atmosphere over 10 minutes. After stirring for 30 minutes, the reaction was quenched by adding water. The aqueous layer was extracted three times with Et_2O. The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and evaporated under reduced pressure to afford **9** (3.90 g, 96%) as a pale yellow oil, without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.59 (1H, d, J = 16.0 Hz), 7.52–7.50 (2H, overlapped), 7.38–7.35 (3H, overlapped), 6.37 (1H, d, J = 16.0 Hz), 1.54 (9H, s). $^{13}C\{^{1}H\}$ NMR (100 MHz, $CDCl_3$) δ 166.3, 143.5, 134.7, 129.9, 128.8, 127.9, 120.2, 80.5, 28.2.

(3S)-β-Hydroxyphenylalanine Derivative ***10***. In reference to the Davies method, **10** was synthesized from **9** over five steps. Data are consistent with literature values.[4] $[\alpha]^{25}_{D}$ +45.3 (*c* 0.65, MeOH); literature value: $[\alpha]^{20}_{D}$ +44.7 (*c* 1.0, MeOH).

(3S)-β-Hydroxyphenylalanine Derivative ***11***. To a solution of **10** (1.43 g, 6.0 mmol) in DCM (12 mL, 0.5 M) was added Boc_2O (1.37 mL, 1.05 eq.) at room temperature under Ar atmosphere. After stirring for 1.5 hours at the same temperature, the reaction was quenched by adding water. The organic layer was washed with water and brine, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was purified by silica gel flash chromatography (AcOEt/hexane = 1:4) to afford **11** as a white solid (2.0 g, quant.): $[\alpha]^{25}_{D}$ +69.8 (*c* 0.97, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35–7.27 (5H, overlapped), 5.32 (1H, d, J = 5.9 Hz), 5.19 (1H, br s), 4.60 (1H, dd, J = 6.6, 3.0 Hz), 4.14 (1H, d, J = 6.9 Hz), 1.45 (9H, s), 1.38 (9H, s); $^{13}C\{^{1}H\}$ NMR (100 MHz, $CDCl_3$) δ 168.6, 156.6, 139.5, 128.1, 127.8, 126.2, 83.7, 80.5, 75.3, 60.2, 28.2, 27.8. Data are consistent with literature values.[5]

*Oxazolidinone **12***. To a solution of **11** (1.23 g, 3.65 mmol) and DIPEA (1.9 mL, 3.0 eq.) in dichloroethane (18.2 mL, 0.2 M) was added dropwise MsCl (0.84 mL, 3.0 eq.) at 0 °C under Ar atmosphere. After stirring for 30 minutes at the same temperature, the reaction mixture was warmed to 60 °C and then stirred for 12.5 hours at the same temperature. The reaction was quenched by adding saturated $NaHCO_3$ aq. The aqueous layer was extracted three times with $CHCl_3$. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The crude residue was used in the next reaction without further purification. To a solution of the above residue in DCM (18.2 mL) were added DMAP (222 mg, 50 mol%) and Boc_2O (0.81 mL, 1.1 eq.) successively at room temperature under Ar atmosphere. After stirring for 1.5 hours, the reaction mixture was washed with saturated citric acid aq. and brine, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was purified by silica gel flash chromatography (AcOEt/hexane = 1:9) to afford **12** as a white solid (927 mg, 70% over 2 steps): $[\alpha]^{24}_D$ +15.0 (*c* 0.47, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47–7.37 (5H, overlapped), 5.33 (1H, d, J = 4.1 Hz), 4.50 (1H, d, J = 5.0 Hz), 1.55 (9H, s), 1.51 (9H, s); ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 167.4, 151.0, 148.3, 137.5, 129.3, 129.1, 124.9, 84.5, 83.8, 76.1, 61.4, 27.9, 27.8. Data are consistent with literature values.[6]

*(3R)-β-Hydroxyphenylalanine Derivative **13***. To a solution of **12** (141.3 mg, 0.39 mmol) in MeOH (3.9 mL, 0.1 M) was added Cs_2CO_3 (38 mg, 0.3 eq.) at room temperature under Ar atmosphere. After stirring for 2 hours at the same temperature, the reaction was quenched by adding 10% citric acid aq. The aqueous layer was extracted three times with $CHCl_3$. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was purified by amino silica gel flash chromatography (MeOH/AcOEt/hexane = 0.1:1:4) to afford **13** as a white solid (105.3 mg, 80%): $[\alpha]^{24}_D$ –9.1 (*c* 1.4, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, VT 55 °C) δ 7.39–7.26 (5H, overlapped), 5.25 (1H, br s), 5.10 (1H, d, J = 3.7 Hz), 4.42 (1H, br s), 2.87 (1H, br s), 1.42 (9H, s), 1.35 (9H, s); ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 169.8, 155.7, 140.0, 128.2, 127.9, 126.3, 82.5, 79.9, 74.5, 59.9, 28.2, 27.9. Data are consistent with literature values.[6]

*(3R)-β-Hydroxyphenylalanine Derivative **14***. To a solution of **13** (436.3 mg, 1.29 mmol) in DCM (13 mL, 0.1 M) was added TFA (4.0 mL, 40 eq.) at 0 °C under Ar atmosphere. After stirring for 1 hour at the same temperature, the reaction was quenched by adding saturated $NaHCO_3$ aq. to pH 8–9. The aqueous layer was extracted three times with $CHCl_3$. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and evaporated under reduced pressure to afford **14** as a colorless solid without further purification (281.8 mg, 92%): $[\alpha]^{22}_D$ +19.4 (*c* 0.66, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37–7.26 (5H, overlapped), 4.73 (1H, d, J = 5.5 Hz), 3.52 (1H, d, J = 5.5 Hz), 1.34 (9H, s); ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 172.5, 140.9, 128.2, 127.8, 126.5, 81.7, 74.6, 61.0, 27.8. Data are consistent with literature values.[18]

*(3R)-β-Hydroxyphenylalanine Derivative **15***. To a solution of **14** (135.0 mg, 0.57 mmol) in THF/DMSO (4:1, 3.0 mL, 0.2 M) were added $NaHCO_3$ (191 mg, 4.0 eq.) and benzyl bromide (0.2 mL, 3.0 eq.) at room temperature under Ar atmosphere. After refluxing at 90 °C for 28 hours, the reaction was quenched by adding water. The aqueous layer was extracted three times with AcOEt. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was purified by silica gel flash chromatography (AcOEt/hexane = 1:9) to afford **15** as a white solid (221.7 mg, 93%): $[\alpha]^{23}_D$ –168.6 (*c* 0.38, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39–7.29 (10H, overlapped), 7.25-7.15 (5H, overlapped), 4.90 (1H, d, *J* = 10.1 Hz), 4.17 (2H, d, *J* = 13.3 Hz), 4.14 (1H, s, OH), 3.51 (2H, d, *J* = 13.3 Hz), 3.30 (1H, d, *J* = 10.1 Hz), 1.37 (9H, s); ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 168.6, 140.3, 138.1, 129.2, 128.6, 128.0, 127.8, 127.5, 81.8, 69.6, 68.0, 54.7, 28.2. Data are consistent with literature values.[19]

*Carboxylic Acid **6** from **15***. TFA (2.2 mL, 0.1 M) was added to **15** (93.0 mg, 0.22 mmol) at room temperature under Ar atmosphere. After stirring for 2 hours, TFA was removed by evaporation and H_2O and DIPEA (1 mL) were added to the residue at 0 °C. The aqueous layer was extracted four times with AcOEt. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was purified by silica gel flash chromatography (AcOH/AcOEt/hexane = 0.1:4:6) to afford **6** as a white amorphous powder (78.8 mg, 98%): $[\alpha]^{26}_D$ –176 (*c* 0.53, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38–7.29 (10H, overlapped), 7.24–7.17 (5H, overlapped), 5.11 (1H, d, *J* = 8.7 Hz), 4.07 (2H, d, *J* = 13.3 Hz), 3.75 (2H, d, *J* = 13.3 Hz), 3.58 (1H, d, *J* = 8.7 Hz); ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 172.4, 139.9, 136.1, 129.4, 128.9, 128.3, 128.2, 128.0, 127.2, 69.3, 67.0, 54.9. Data are consistent with literature values.[19]

*Oxazolidinone **16***. AcCl (0.4 mL) was added dropwise to MeOH (2.0 mL, 0.5 M) at 0 °C under Ar atmosphere. After stirring for 5 minutes, L-phenylalanine (165.2 mg, 1.0 mmol) was added to the solution. The reaction mixture was refluxed at 80 °C for 3 hours. After cooling to room temperature, the reaction was quenched by adding 30% NH_3 aq. The aqueous layer was extracted three times with $CHCl_3$. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The crude residue was used in the next reaction without further purification. To a solution of the above residue in THF (2.0 mL) was added Boc_2O (218.3 μL, 1.0 eq.) at room temperature under Ar atmosphere. After stirring for 1 hour at the same temperature, the reaction mixture was diluted with AcOEt. The organic layer was washed with water and brine, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The crude residue was used in the next reaction without further purification. To a solution of the above residue in THF (5.0 mL) was added dropwise a 3 M MeMgBr solution in Et_2O (1.3 mL, 4.0 eq.) at 0 °C under Ar atmosphere. After stirring for 15 minutes at the same temperature, the reaction mixture was allowed to warm to room temperature and stirred for a total of 3 hours. The reaction was quenched by adding saturated NH_4Cl aq. The aqueous layer was extracted three

times with AcOEt. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The crude residue obtained by purification by silica gel short-column flash chromatography was used in the next reaction without further purification. To a solution of the above residue in THF (3.0 mL) was added KO*t*-Bu (118.3 mg, 1.2 eq.) at room temperature under Ar atmosphere. After stirring for 45 minutes at the same temperature, the reaction was quenched by adding 10% citric acid aq. to pH 4–5. The aqueous layer was extracted two times with AcOEt. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was purified by silica gel flash chromatography (AcOEt/hexane = 3:7 to 1:1 gradient) to afford **16** as a pale yellow solid (170.8 mg, 83.2% over 4 steps): $[\alpha]^{22}_D$ –102.4 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) 7.36–7.32 (2H, overlapped), 7.29–7.25 (1H, m), 7.19–7.17 (2H, overlapped), 5.28 (1H, br s), 3.69 (1H, ddd, *J* = 10.8, 3.6, 0.8 Hz), 2.84 (1H, dd, *J* = 13.2, 3.6 Hz), 2.59 (1H, br dd, *J* = 13.2, 10.8 Hz), 1.48 (3H, s), 1.46 (3H, s); ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 158.0, 136.8, 128.9, 128.7, 127.0, 83.1, 62.9, 36.9, 27.4, 21.8. Data are consistent with literature values.[7]

*Bromoacetyl Oxazolidinone **17***. To a solution of **16** (170.8 mg, 0.83 mmol) in THF (2.8 mL, 0.1 M) was added dropwise a 1.55 M *n*-BuLi solution in hexane (0.59 mL, 1.1 eq.) at –78 °C under Ar atmosphere and the mixture was stirred for 10 minutes at the same temperature. After the addition of 2-bromoacetyl bromide (86.3 μL, 1.2 eq.), the reaction mixture was allowed to warm to room temperature and stirred for 1.5 hours. The reaction was quenched by adding 10% citric acid aq. The aqueous layer was extracted once with AcOEt. The organic layer was washed with saturated $NaHCO_3$ aq. and brine, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was purified by silica gel flash chromatography (AcOEt/hexane = 1:9) to afford **17** as a pale yellow oil (206.7 mg, 76%): $[\alpha]^{22}_D$ –27.8 (*c* 0.71, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) 7.35–7.23 (5H, overlapped), 4.58 (1H, d, *J* = 12.4 Hz), 4.51 (1H, dd, *J* = 9.6, 3.7 Hz), 4.44 (1H, d, *J* = 12.4 Hz), 3.19 (1H, dd, *J* = 14.4, 3.7 Hz), 2.59 (1H, br dd, *J* = 14.4, 9.6 Hz), 1.40 (3H, s), 1.39 (3H, s); ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) 166.6, 152.1, 136.4, 129.0, 128.7, 126.9, 83.3, 64.0, 34.9, 28.6, 28.3, 21.8. Data are consistent with literature values.[8]

*Evans-aldol Reaction Substrate **18***. To a solution of **17** (2.61 g, 8.0 mmol) in THF (26 mL, 0.3 M) were added DIPEA (1.68 mL, 1.2 eq.) and Bn_2NH (1.84 mL, 1.2 eq.) at room temperature under Ar atmosphere. The reaction mixture was warmed to 40 °C and stirred for 4 hours at the same temperature. The reaction mixture was diluted with AcOEt and the organic layer was washed with water and brine, dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The residue was purified by silica gel flash chromatography (AcOEt/hexane = 1:9 to 3:7 gradient) to afford **18** as a colorless oil (3.42 g, 97%): $[\alpha]^{22}_D$ –16.2 (*c* 1.08, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) 7.38–7.37 (4H, overlapped), 7.31–7.22 (11H, overlapped), 4.50 (1H, dd, *J* = 9.6, 4.1 Hz), 3.94 (1H, d, *J* = 18.6 Hz), 3.86 (1H, d, *J* = 18.6 Hz), 3.81 (4H, d, *J* = 13.7 Hz), 3.13 (1H, dd, *J* = 14.4, 4.1 Hz), 2.86 (1H, dd, *J* = 14.4, 9.6 Hz), 1.35 (3H, s), 1.32 (3H, s); ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) 171.7, 152.5, 139.2, 136.8, 129.1, 128.8, 128.7, 128.3, 127.1, 126.8,

82.8, 63.1, 57.5, 55.4, 35.3, 28.4, 22.2. Data are consistent with literature values.[8]

*Oxazolidinone **19***. To a solution of **18** (468.5 mg, 1.1 mmol) in DCM (11 mL, 0.1 M) was added freshly prepared 1 M 9-BBNOTf solution in toluene (1.32 mL, 1.2 eq.) at 0 °C under Ar atmosphere. After stirring for 10 minutes, DIPEA (0.27 mL, 1.4 eq.) was added to the reaction mixture and this was stirred for 20 minutes at the same temperature. After the addition of fresh benzaldehyde (122 mL, 1.1 eq.) at –78 °C, the reaction mixture was stirred for 30 minutes and allowed to warm to 0 °C. The reaction was quenched by adding MeOH/30% H_2O_2 (1:1) and stirred for 30 minutes at room temperature. The aqueous layer was extracted three times with $CHCl_3$. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was purified by silica gel flash chromatography (AcOEt/hexane = 1:9) to afford **18** (65.0 mg, 13% recovered) and **19** as a white solid (516.7 mg, 86%): $[\alpha]^{22}_D$ –36.1 (*c* 1.06, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) 7.39–7.17 (20H, overlapped), 5.25 (1H, d, *J* = 9.6 Hz), 4.77 (1H, d, *J* = 9.6 Hz), 4.30 (1H, dd, *J* = 7.8, 6.6 Hz), 4.03 (2H, d, *J* = 14.2 Hz), 3.84 (1H, s), 3.43 (2H, d, *J* = 14.2 Hz), 2.85 (1H, dd, *J* = 14.2, 7.8 Hz), 2.80 (1H, dd, *J* = 14.2, 6.6 Hz), 1.26 (3H, s), 0.58 (3H, s); ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) 169.9, 151.8, 139.0, 138.7, 136.5, 129.4, 129.1, 128.7, 128.4, 128.3, 128.1, 127.3, 127.0, 81.9, 71.9, 66.3, 62.7, 54.6, 36.1, 26.9, 21.7. Data are consistent with literature values.[8]

The 1 M 9-BBNOTf solution in toluene was prepared as follows. 9-BBN dimer (671 mg, 2.75 mmol) was added into a vial equipped with a stirrer bar under Ar atmosphere in a glove box. The vial was sealed with a septum and then toluene (5 mL, 1.0 M) and TfOH (0.44 mL, 5 mmol) were added using a syringe. The color of the reaction mixture changed from colorless to red. After the reaction mixture was refluxed at 120 °C for 1 hour, its color became pale yellow. The reaction mixture was cooled to room temperature and used in the next reaction without further purification.

*Carboxylic Acid **6** from **19***. To a solution of **19** (1.0 g, 1.82 mmol) in THF/H_2O (4:1, 18 mL, 0.1 M) were added 30% H_2O_2 aq. (0.37 mL, 2.0 eq.) and 0.8 M LiOH aq. (4.5 mL, 2.0 eq.) at 0 °C under Ar atmosphere. The reaction mixture was stirred for 30 minutes and then allowed to warm to room temperature. After stirring for 20 hours at the same temperature, the reaction was quenched by adding saturated Na_2SO_3 aq. and stirred for 1 hour. After the addition of AcOH to the solution to pH 4–5, the aqueous layer was extracted three times with AcOEt. The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The residue was purified by silica gel flash chromatography (5% AcOH-AcOEt/$CHCl_3$ = 1:9) to afford **6** as a white amorphous powder (419.5 mg, 64%). Data are consistent with those of carboxylic acid **6** from **15**.

*4-Fluoro-3-nitrobenzyl Bromide (**21**)*. To a solution of conc. H_2SO_4 (24 mL) and conc. HNO_3 (3 mL) was added dropwise 4-fluorobenzaldehyde (**20**) (5 mL, 47.5 mmol) at –5 °C under open air. After the addition of **20**, the reaction mixture was allowed to warm to room temperature over 1 hour. The reaction mixture was slowly added to cold water containing ice and the aqueous layer was extracted three times

with DCM. The combined organic layers were washed with saturated $NaHCO_3$ aq. and brine, dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The residue was purified by silica gel flash chromatography (Et_2O/hexane = 1:3 to 3:7 gradient) to afford 4-fluoro-3-nitrobenzaldehyde as a pale yellow solid (5.28 g, 66%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.04 (1H, s), 8.60 (1H, dd, J = 6.8, 1.6 Hz), 8.20 (1H, ddd, J = 8.8, 4.0, 2.4 Hz), 7.50 (1H, dd, J = 10.0, 8.8 Hz). Data are consistent with literature values.[20] To a solution of 4-fluoro-3-nitrobenzaldehyde (5.26 g, 31.1 mmol) in MeOH (78 mL, 0.4 M) was added $NaBH_4$ (705 mg, 0.6 eq.) in one portion at 0 °C under Ar atmosphere. The reaction mixture was allowed to warm to room temperature and stirred for 20 minutes at the same temperature. The reaction mixture was concentrated by evaporation and diluted with water. The aqueous layer was extracted three times with $CHCl_3$. The combined organic layers were dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The residue was purified by silica gel short-column flash chromatography (AcOEt) to afford 4-fluoro-3-nitrobenzyl alcohol as a pale yellow solid (5.19 g, 98%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 (1H, dd, J = 6.9, 2.3 Hz), 7.63 (1H, ddd, J = 8.7, 6.4, 2.3 Hz), 7.28 (1H, dd, J = 10.5, 8.7 Hz), 4.75 (2H, d, J = 5.0 Hz), 2.10 (1H, t, J = 5.5 Hz, OH); ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 154.7 (d, J = 265 Hz), 137.9 (d, J = 3.8 Hz), 137.2 (d, J = 6.7 Hz), 133.6 (d, J = 8.6 Hz), 124.0 (d, J = 2.9 Hz), 118.5 (d, J = 21 Hz), 63.2. Data are consistent with literature values.[9] To a solution of 1,2-bis(diphenylphosphino)ethane (7.24 g, 0.6 eq.) in DCM (61 mL) was added a 6.25 M Br_2 solution in DCM (7.3 mL, 1.5 eq.) using a syringe at –20 °C under Ar atmosphere. After stirring for 15 minutes, a solution of 4-fluoro-3-nitrobenzyl alcohol (5.19 g, 30.5 mmol) in DCM (60 mL) was added via a cannula to the reaction mixture at the same temperature. After the reaction mixture was stirred for 2 hours at the same temperature, it was allowed to warm to room temperature and stirred overnight. The reaction mixture was diluted with 50% Et_2O/hexane (120 mL) and filtered through a pad of Celite® with Et_2O/hexane (2:1, 50 mL × 2), and the filtrate was concentrated under reduced pressure. The residue was purified by silica gel flash chromatography (AcOEt/hexane = 1:1) to afford 4-fluoro-3-nitrobenzyl bromide (**21**) as a yellow solid (6.82 g, 96%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.11 (1H, dd, J = 6.9, 2.3 Hz), 7.67 (1H, ddd, J = 8.5, 6.9, 2.3 Hz), 7.29 (1H, dd, J = 10.5, 8.7 Hz), 4.49 (2H, s); ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 155.1 (d, J = 267 Hz), 137.1 (d, J = 8.6 Hz), 136.0 (d, J = 8.6 Hz), 135.0 (d, J = 3.8 Hz), 126.5 (d, J = 1.9 Hz), 119.0 (d, J = 22 Hz), 30.3. Data are consistent with literature values.[9]

*4-Fluoro-3-nitrobenzyl Iodine (**22**)*. To a solution of **21** (1.52 g, 6.5 mmol) in acetone (13 mL, 0.5 M) was added NaI (1.17 g, 1.2 eq.) at room temperature. The reaction mixture was stirred for 20 minutes at the same temperature under Ar atmosphere. The reaction mixture was quenched with cold water and extracted two times with AcOEt. The combined organic layers were washed two times with brine, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was purified by recrystallization from 20%AcOEt/hexane to afford 4-fluoro-3-nitrobenzyl iodine **22** as pale yellow needles (1.65 g, 90%): m.p.; 80–81 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 (1H, dd, J = 6.9, 2.3 Hz), 7.64

(1H, ddd, J = 8.5, 6.9, 2.3 Hz), 7.25 (1H, dd, J = 10.5, 8.7 Hz), 4.44 (2H, s); $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$) δ 154.6 (d, J = 267 Hz), 137.2 (d, J = 6.7 Hz), 136.7 (d, J = 3.8 Hz), 135.7 (d, J = 8.6 Hz), 126.0 (d, J = 2.9 Hz), 119.0 (d, J = 21 Hz), 1.1. Data are consistent with literature values.[10]

*Alkylated Compound **23***. CuCN (423.2 mg, 1.5 eq.) was added into a vial equipped with a stirrer bar and dried well over 1 hour under reduced pressure. The residue was dissolved in THF (20 mL) and the solution was cooled to 0 °C with stirring. To a solution of Schöllkopf reagent (870.3 mg, 1.5 eq.) in THF (7 mL) in another vial was added 1.55 M *n*-BuLi solution in hexane (3.05 mL, 1.5 eq.) at –78 °C under Ar atmosphere. After stirring for 10 minutes, this solution was added to the other vial via a cannula. After the reaction mixture was stirred for 10 minutes at 0 °C, it was cooled to –78 °C. To the reaction mixture was added a solution of **22** (884.8 mg, 3.15 mmol) in THF (3 mL) via a cannula. The reaction mixture was rinsed with THF (2 mL) at the same temperature over 5 minutes and stirred for 15 minutes. The reaction was quenched by adding saturated NH_4Cl aq./30% NH_4OH aq. (9:1) at –78 °C and allowed to warm to room temperature. The aqueous layer was extracted three times with AcOEt. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was purified by silica gel flash chromatography (AcOEt/hexane = 1:9) to afford alkylated compound **23** as a pale yellow oil (871.2 mg, 82%): $[\alpha]^{25}_D$ +37.7 (*c* 0.46, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 7.91 (1H, dd, J = 7.6, 2.1 Hz), 7.38 (1H, m), 7.14 (1H, dd, J = 10.7, 8.6 Hz), 4.29 (1H, dd, J = 9.6, 4.1 Hz), 3.73 (3H, s), 3.69 (3H, s), 3.56 (1H, dd, J = 3.4, 3.4 Hz), 3.17 (1H, dd, J = 13.4, 4.5 Hz), 3.13 (1H, dd, J = 13.7, 5.5 Hz), 2.17 (1H, qd, J = 6.9, 3.4 Hz), 0.97 (3H, d, J = 6.9 Hz), 0.64 (3H, d, J = 6.9 Hz); $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$) δ 164.4, 161.7, 154.3 (d, J = 264 Hz), 136.9 (d, J = 7.7 Hz), 136.5 (d, J = 6.7 Hz), 134.8 (d, J = 3.8 Hz), 127.4 (d, J = 2.9 Hz), 117.6 (d, J = 21 Hz), 60.7, 55.8, 52.5, 52.4, 38.6, 31.7, 18.9, 16.5; HRMS (ESI) m/z: $[M+H]^+$ calcd for $C_{16}H_{21}FN_3O_4^+$ 338.1516, found 338.1538; IR (ATR) ν_{max} 1695, 1538, 1351, 1241, 1015 cm^{-1}.

*Chiral Phenylalanine Derivative **8***. To a solution of **23** (101.5 mg, 0.30 mmol) in acetonitrile (1.5 mL, 0.2 M) was added 0.25 N HCl aq. (3.0 mL, 2.5 eq.) at room temperature under Ar atmosphere. After stirring for 3 hours at the same temperature, the reaction was quenched by adding saturated $NaHCO_3$ aq. The aqueous layer was extracted four times with $CHCl_3$. The combined organic layers were dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was purified by silica gel flash chromatography (MeOH/AcOEt/$CHCl_3$ = 3:30:70) to afford chiral phenylalanine derivative **8** as a yellow oil (70.5 mg, 97%). As compound **8** easily formed a dimer under concentrated condition, it was used immediately in the next reaction. Otherwise, it should be stored as an HCl salt. $[\alpha]_D^{25}$ +13.0 (*c* 0.4, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 7.93 (1H, dd, J = 7.1, 2.2 Hz), 7.49 (1H, m), 7.22 (1H, dd, J = 10.8, 8.5 Hz), 3.73 (3H, s), 3.71 (1H, dd, J = 7.8, 5.0 Hz), 3.10 (1H, dd, J = 13.7, 5.0 Hz), 2.91 (1H, dd, J = 13.7, 7.8 Hz), 1.55 (2H, br s, NH_2); $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$) δ 174.9, 154.4 (d, J = 264 Hz), 137.1 (d, J = 7.7 Hz), 136.5 (d, J = 8.6 Hz), 134.7 (d, J = 4.8 Hz), 126.6 (d, J = 2.9 Hz), 118.3 (d, J = 21

Hz), 55.4, 52.3, 52.4, 39.6. Data are consistent with literature values.[13]

*Dipeptide **24***. To a solution of **8** (68.3 mg, 0.28 mmol) in DCM (1.4 mL, 0.2 M) were added *N*-Boc-L-phenylalanine (89 mg, 1.3 eq.), DIPEA (54 μL, 1.1 eq.), HOBt (48 mg, 1.1 eq.), and EDCI (60 mg, 1.1 eq.) successively at room temperature. After stirring for 2 hours under Ar atmosphere, the reaction was quenched by adding 10% citric acid aq. The aqueous layer was extracted three times with $CHCl_3$. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was purified by silica gel flash chromatography (AcOEt/hexane = 1:1) to afford dipeptide **24** as a pale yellow solid (123.6 mg, 90%): $[\alpha]^{25}_D$ +36.5 (*c* 1.07, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, VT 55 °C) δ 7.71 (1H, dd, *J* = 6.9, 2.3 Hz), 7.33–7.12 (7H, overlapped), 6.45 (1H, d, *J* = 6.9 Hz, N*H*COO), 4.90 (1H, d, *J* = 7.3 Hz, N*H*CO), 4.77 (1H, dd, *J* = 13.3, 6.0 Hz), 4.31 (1H, dd, *J* = 14.6, 6.9 Hz), 3.69 (3H, s), 3.18 (1H, dd, *J* = 14.2, 6.0 Hz), 3.08-3.03 (3H, overlapped), 1.40 (9H, s); ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 171.2, 170.6, 155.4, 154.6 (d, *J* = 265 Hz), 137.0 (d, *J* = 6.7 Hz), 136.5 (d, *J* = 8.6 Hz), 136.2, 133.0 (d, *J* = 3.8 Hz), 129.2, 128.7, 127.0, 126.6 (d, *J* = 2.9 Hz), 118.5 (d, *J* = 20 Hz), 80.5, 55.9, 52.9, 52.7, 37.8, 36.7, 28.1; HRMS (ESI) m/z: $[M+Na]^+$ calcd for $C_{24}H_{28}FN_3NaO_7^+$ 512.1809, found 512.1839; IR (ATR) ν_{max} 3332, 3301, 1740, 1663, 1540, 1521, 1352, 1295, 1250, 1167 cm^{-1}.

*Tripeptide **5***. To a solution of **24** (161.9 mg, 0.33 mmol) in DCM (1.7 mL, 0.2 M) was added TFA (0.76 mL, 30 eq.) at 0 °C under Ar atmosphere. After stirring for 2 hours at the same temperature, the reaction was quenched by adding saturated $NaHCO_3$ aq. to pH 8–9 and diluted with water. The aqueous layer was extracted three times with $CHCl_3$. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and evaporated under reduced pressure for 10 minutes to afford a yellow solid residue. As the residue easily formed a dimer, it was used immediately in the next reaction without further purification. To a solution of the above residue (129.6 mg, <0.33 mmol) in MeOH/acetonitrile (1:1, 6.6 mL) were added a solution of **6** (131.5 mg, 1.1 eq.) in MeOH/acetonitrile (1:1, 1.4 mL), DIPEA (86.5 μL, 1.5 eq.), and DMT-MM (137.0 mg, 1.5 eq.) at room temperature. After stirring for 2 hours at the same temperature under Ar atmosphere, the reaction was quenched by adding 10% citric acid aq. The aqueous layer was extracted three times with AcOEt. The combined organic layers were washed with saturated $NaHCO_3$ aq. and brine, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was purified by silica gel flash chromatography (AcOEt/hexane = 2:3) to afford tripeptide **5** as a pale yellow amorphous powder (226.3 mg, 93% over 2 steps): $[\alpha]^{24}_D$ –103.8 (*c* 0.46, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38–7.26 (10H, overlapped), 7.22–7.17 (12H, overlapped), 7.11 (1H, dd, *J* = 10.5, 8.2 Hz), 6.26 (1H, d, *J* = 7.8 Hz, N*H*CO), 5.90 (1H, d, *J* = 7.8 Hz, N*H*CO), 5.08 (1H, d, *J* = 9.2 Hz), 4.71 (1H, m), 4.61 (1H, ddd, *J* = 7.3, 5.5, 5.5 Hz), 4.09 (1H, br s, OH), 3.98 (2H, d, *J* = 13.7 Hz), 3.75 (3H, s), 3.16 (1H, d, *J* = 8.7 Hz), 3.11 (1H, dd, *J* = 14.4, 6.4 Hz), 3.08 (2H, d, *J* = 13.7 Hz), 3.00 (1H, dd, *J* = 13.7, 5.5 Hz), 2.91 (1H, dd, *J* = 14.4, 8.9 Hz), 2.76 (1H, dd, *J* = 13.7, 6.9 Hz); ^{13}C{^{1}H} NMR (100 MHz,

$CDCl_3$) δ 170.4, 170.1, 169.1, 154.4 (d, J = 265 Hz), 140.7, 138.1, 137.0 (d, J = 8.6 Hz), 136.4 (d, J = 7.7 Hz), 136.1, 133.1 (d, J = 3.8 Hz), 129.1,129.0, 128.8 128.75, 128.3, 127.9, 127.6, 127.4, 126.4 (d, J = 1.9 Hz), 118.4 (d, J = 21 Hz), 70.2, 67.9, 54.4, 53.8, 53.0, 52.7, 37.3, 36.9; HRMS (ESI) m/z: $[M+H]^+$ calcd for $C_{42}H_{42}FN_4O_7^+$ 733.3038, found 733.3073; IR (ATR) ν_{max} 3393 (br), 1745, 1667, 1539, 1496, 1454, 1354, 1251, 1217, 753, 702 cm^{-1}.

*14-Membered Paracyclophanes **25a** and **25b***. A suspension of 1.0 M TBAF solution in THF (0.95 mL, 1.1 eq.) and powdered 4Å molecular sieves (420 mg, pre-dried under vacuum) in DMF (138 mL) was stirred over 2 hours at room temperature. To the suspension was added a solution of **5** (629.3 mg, 0.86 mmol) in DMF (34 mL) via a cannula over 25 minutes at the same temperature under Ar atmosphere. The reaction mixture was warmed to 70 °C and stirred for 27 hours. After cooling to room temperature, the reaction mixture was filtered through a pad of Celite® with AcOEt and the filtrate was diluted with water and extracted four times with AcOEt. The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The residue was purified by silica gel flash chromatography (AcOEt/hexane = 2:3) to afford a mixture of inseparable atropisomers, **25a** and **25b**, as a pale yellow solid (268.9 mg, 44%, in the ratio of 5:1): $[\alpha]^{24}_D$ –134.8 (*c* 0.84, $CHCl_3$, **25a**:**25b** = 5:1); ^1H NMR (600 MHz, $CDCl_3$) δ 7.67 (1H, d, J = 2.1 Hz, H-13), 7.43–7.41 (3H, overlapped), 7.35–7.31 (5H, overlapped), 7.20–7.17 (3H, overlapped), 7.13–7.12 (6H, overlapped), 6.91 (1H, d, J = 8.9 Hz, H-14), 6.65–6.64 (4H, overlapped, Bn), 5.72 (1H, d, J = 4.8 Hz, H-3), 5.63 (1H, d, J = 8.9 Hz, NH), 5.50 (1H, d, J = 11.0 Hz, NH), 5.01 (1H, ddd, J = 11.7, 11.0, 6.9 Hz, H-10), 4.53 (1H, ddd, J = 8.9, 7.6, 7.6 Hz, H-7), 3.75 (3H, s, CO_2Me), 3.48 (1H, dd, J = 13.7, 6.8 Hz, H-11a), 3.38 (1H, d, J = 4.8 Hz, H-4), 2.87 (1H, dd, J = 14.4, 8.2 Hz, H-27), 2.82 (1H, dd, J = 14.4, 7.6 Hz, H-27), 2.60 (1H, dd, J = 13.7, 11.7 Hz, H-11b); $^{13}C\{^1H\}$ NMR (150 MHz, $CDCl_3$) δ 170.7 (C-34), 170.1 (C-8), 169.0 (C-4), 153.9 (C-1), 141.9, 136.9, 135.4, 135.3, 130.0, 129.1, 128.9, 128.7, 128.3, 127.9, 127.8, 127.6, 127.3, 127.2, 126.6, 125.8, 120.1, 88.2 (C-3), 65.1 (C-4), 54.7, 52.8, 52.3, , 51.9, 39.9, 37.8; HRMS (ESI) m/z: $[M+H]^+$ calcd for $C_{42}H_{41}N_4O_7^+$ 713.2975, found 713.2937; IR (ATR) ν_{max} 3393, 1745, 1667, 1539, 1496, 1454, 1354, 1251, 1217, 753, 702 cm^{-1}.

*14-Membered Cyclopeptide Core **26***. To a solution of a mixture of **25a** and **25b** (2.4 mg, 3.4 μmol) in H_2O/EtOH (1:2, 0.34 mL, 0.01 M) were added Fe powder (2.0 mg, 10 eq.) and NH_4Cl (0.55mg, 3 eq.) at room temperature. After refluxing for 1 hour at 80 °C under Ar atmosphere, the reaction mixture was cooled to room temperature and diluted with water. The aqueous layer was extracted five times with AcOEt. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue (2.3 mg) was filtered through a pad of standard Al_2O_3 silica and then used in the next reaction immediately without further purification. To a solution of the above residue (2.1 mg, <3.1 μmol) in THF (0.3 mL, 0.01 M) were added 0.63 M H_3PO_2 solution in THF (0.1 mL, 20 eq.) and 0.2 M *tert*-BuONO solution in THF (46 μL, 3 eq.) at 0 °C under Ar atmosphere.

After stirring for 5 minutes, the reaction mixture was allowed to warm to room temperature and stirred for 1 hour. The reaction mixture was diluted with AcOEt, washed with water, saturated $NaHCO_3$ aq., and brine, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was purified by silica gel preparative thin-layer chromatography (AcOEt/hexane = 2:3) to afford **26** as a white solid (2.0 mg, 89% over 2 steps): $[\alpha]^{23}_D$ –42.2 (*c* 0.49, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.39–7.36 (3H, overlapped), 7.33–7.26 (2H, overlapped), 7.26 (1H, m), 7.19–7.10 (12H, overlapped), 6.86 (1H, dd, *J* = 8.2, 2.1 Hz), 6.74–6.72 (5H, overlapped), 5.65 (1H, d, *J* = 8.2 Hz), 5.55 (1H, d, *J* = 4.1 Hz), 5.04 (1H, d, *J* = 11.0 Hz), 4.90 (1H, ddd, *J* = 11.7, 11.0, 6.2 Hz), 4.36 (1H, ddd, *J* = 8.2, 7.6, 6.9 Hz), 3.74 (3H, s), 3.42 (1H, d, *J* = 4.1 Hz), 3.40 (1H, dd, *J* = 13.1, 6.2 Hz), 2.84 (1H, dd, *J* = 13.7, 7.6 Hz), 2.80 (1H, dd, *J* = 13.7, 6.9 Hz), 2.48 (1H, dd, *J* = 13.1, 11.7 Hz); ^{13}C{^{1}H} NMR (150 MHz, $CDCl_3$) δ 171.2, 169.9, 169.4, 160.1, 138.6, 135.9, 131.5, 130.8, 130.3, 129.3, 128.6, 128.0, 127.8, 127.2, 127.1, 125.9, 121.5, 118.4, 87.7, 64.5, 55.3, 53.3, 53.1, 52.5, 40.4, 38.6; HRMS (ESI) m/z: $[M+H]^+$ calcd for $C_{42}H_{42}N_3O_5^+$ 668.3125, found 668.3116; IR (ATR) ν_{max} 3358, 3314, 3286, 3061, 3028, 1733, 1653, 1511, 1496, 1451, 1365, 1232, 755, 700 cm^{-1}.

*Primary Amine **3***. To a solution of **26** (13.2 mg, 0.02 mmol) in MeOH/DCM (1:1, 1.0 mL, 0.02 M) was added Pd/C (10%, 6.3 mg, 30 mol%) at room temperature. The reaction mixture was stirred for 3.5 h at room temperature under hydrogen atmosphere. The reaction mixture was filtered through a pad of Celite® with MeOH. The filtrate was evaporated under reduced pressure to afford **3** as a white solid without further purification (10.1 mg, quant.): $[\alpha]^{23}_D$ –32.5 (*c* 1.23, MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 7.62 (2H, d, *J* = 7.6 Hz), 7.48 (2H, dd, *J* = 7.6, 7.6 Hz), 7.40 (1H, dd, *J* = 7.6 Hz), 7.23–7.12 (6H, m), 6.95 (1H, dd, *J* = 8.6, 2.1 Hz), 6.82 (1H, dd, *J* = 8.2, 2.1 Hz), 6.86 (1H, dd, *J* = 8.6, 2.1 Hz), 5.76 (1H, br s), 4.87 (1H, m), 4.36 (1H, dd, *J* = 7.9, 7.6 Hz), 3.87 (1H, br s), 3.69 (3H, s) , 3.36 (1H, dd, *J* = 13.4, 5.8 Hz), 2.85 (1H, dd, *J* = 13.7, 7.6 Hz), 2.67 (1H, dd, *J* = 13.7, 7.9 Hz), 2.59 (1H, dd, *J* = 13.4, 11.7 Hz); ^{13}C{^{1}H} NMR (150 MHz, CD_3OD) δ 172.6, 171.4, 159.4, 137.7, 134.1, 133.0, 131.6, 130.4, 130.2, 129.8, 129.4, 127.8, 126.8, 122.6, 120.2, 84.5, 59.1, 55.5, 54.2, 52.8, 40.7, 38.3; HRMS (ESI) m/z: $[M+H]^+$ calcd for $C_{28}H_{30}N_3O_5^+$ 488.2186, found 488.2209; IR (ATR) ν_{max} 3355, 3210, 3037, 2955, 1734, 1667, 1608, 1558, 1508, 1456, 1285, 1223, 1173, 1013, 702 cm^{-1}.

N,N-Dimethyl-O-benzyltyrosine Methyl Ester. AcCl (0.8 mL) was added dropwise to MeOH (4.0 mL, 0.5 M) at 0 °C under Ar atmosphere. After the solution was stirred for 5 minutes, L-tyrosine (362.4 mg, 2.0 mmol) was added to the solution. The reaction mixture was refluxed at 80 °C for 1 hour. After cooling to room temperature, the reaction was quenched by adding 30% NH_3 aq. The aqueous layer was extracted four times with $CHCl_3$. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The crude residue was used in the next reaction without further purification. The above residue was dissolved in MeOH (2.0 mL) and Pd/C (10 mol%) and formalin (2.5 eq., 36%) were added to the solution at room temperature. The reaction mixture was stirred for 1 hour

under hydrogen atmosphere. The reaction mixture was filtered through a pad of Celite® with MeOH. The filtrate was purified through an amino silica gel pad with MeOH/$CHCl_3$ (1:1) to afford *N*,*N*-dimethyltyrosine methyl ester as a white solid. This was used in the next reaction without further purification. To a solution of *N*,*N*-dimethyltyrosine methyl ester (111.7 mg, 0.5 mmol) in DMF (2.5 mL, 0.2 M) was added NaH (20 mg, 1.0 eq., 60%) at 0 °C. After stirring for 10 minutes at the same temperature under Ar atmosphere, BnBr (59.4 μL, 1.0 eq.) was added dropwise to the solution. The reaction mixture was allowed to warm to room temperature and stirred for 1.5 hours. The reaction was quenched by adding water. The aqueous layer was extracted three times with AcOEt. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was purified by silica gel flash chromatography (AcOEt/hexane = 2:3) to afford *N*,*N*-dimethyl-*O*-benzyltyrosine methyl ester as a colorless oil (150.3 mg, 96%): $[\alpha]^{25}_D$ +25.1 (*c* 0.92, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43–7.30 (5H, overlapped), 7.12–7.09 (2H, overlapped), 6.89–6.88 (2H, overlapped), 5.02 (2H, s), 3.60 (3H, s), 3.37 (1H, dd, *J* = 9.6, 5.5 Hz), 2.99 (1H, dd, *J* = 13.7, 9.6 Hz), 2.87 (1H, dd, *J* = 13.7, 5.5 Hz), 2.38 (6H, s); ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 171.9, 157.4, 137.0, 130.3, 130.0, 128.5, 127.9, 127.5, 114.7, 69.9, 69.8, 51.0, 41.9, 34.9; HRMS (ESI) m/z: $[M+H]^+$ calcd for $C_{19}H_{24}N_1O_3^+$ 314.1756, found 314.1744; IR (ATR) ν_{max} 2948, 2786, 1731, 1611, 1510, 1455, 1382, 1297, 1240, 1164, 1025, 826, 738, 696, 629 cm^{-1}.

*N,N-Dimethyl-O-benzyltyrosine **4***. To a solution of *N*,*N*-dimethyl-*O*-benzyltyrosine methyl ester (40.5 mg, 0.13 mmol) in THF/H_2O (2:1, 1.0 mL, 0.13 M) was added LiOH•H_2O (8 mg, 1.5 eq.) at room temperature. After stirring for 9 hours at 50 °C, the reaction was quenched b adding 1 N HCl aq. to pH 1–2. The aqueous layer was extracted four times with 10% MeOH/$CHCl_3$. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was purified by silica gel flash chromatography (5% AcOH-MeOH/$CHCl_3$ = 1:4) to afford *N*,*N*-dimethyl-*O*-benzyltyrosine **4** as a white amorphous powder (30.8 mg, 80%): $[\alpha]^{26}_D$ +41.8 (*c* 0.79, MeOH); ^{1}H NMR (400 MHz, CD_3OD) δ 7.43 (2H, overlapped), 7.35 (2H, overlapped), 7.29 (1H, m), 7.24 (2H, overlapped), 6.97 (2H, overlapped), 5.07 (2H, s), 4.27 (1H, dd, *J* = 8.2, 6.0 Hz), 3.35 (1H, dd, *J* = 14.2, 6.0 Hz), 3.19 (1H, dd, *J* = 14.2, 8.2 Hz), 2.96 (6H, s); ^{13}C{^{1}H} NMR (100 MHz, CD_3OD) δ 170.1, 159.8, 138.6, 131.5, 129.5, 128.9, 128.5, 127.7, 116.4, 71.0, 70.0, 42.4, 33.8. Data are consistent with literature values.[16]

*Cyclophane **2***. To a solution of **3** (14.9 mg, 0.031 mmol) in MeOH/DCM (1:1, 0.8 mL, 0.04 M) were added **4** (11.1 mg, 1.2 eq.), DIPEA (22 μL, 4 eq.), and DMT-MM (17.2 mg, 2 eq.) at room temperature. After stirring for 4 hours at the same temperature under Ar atmosphere, the reaction was quenched by adding 10% citric acid aq. The aqueous layer was extracted three times with $CHCl_3$. The combined organic layers were washed with saturated $NaHCO_3$ aq. and brine, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was purified by amino silica gel flash chromatography

(AcOEt/hexane = 4:1) to afford cyclophane **2** as a white solid (20.3 mg, 86% over 2 steps): $[\alpha]^{25}_{D}$ +8.0 (*c* 0.37, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.69 (1H, br d, *J* = 9.0 Hz, NH-21), 7.52 (2H, d, *J* = 7.8 Hz, H-18), 7.43–7.40 (4H, overlapped, H-19, Bn), 7.37 (2H, dd, *J* = 7.8, 7.8 Hz, Bn), 7.32-7.29 (2H, overlapped), 7.24–7.18 (3H, overlapped), 7.15 (1H, dd, *J* = 8.4, 2.4 Hz, H-13), 7.06 (2H, d, *J* = 7.2 Hz, H-31), 6.97 (1H, dd, *J* = 8.4, 2.4 Hz, H-14), 6.88 (1H, dd, *J* = 8.4, 2.4 Hz, H-15), 6.87 (2H, d, *J* = 8.4 Hz, H-26), 6.80 (1H, dd, *J* = 8.4, 2.4 Hz, H-16), 6.79 (2H, d, *J* = 8.4 Hz, H-27), 6.09 (1H, d, *J* = 8.2 Hz, NH-6), 5.63 (1H, s, H-3), 5.02–4.98 (3H, overlapped, H-9, Bn), 4.89–4.85 (2H, overlapped, H-4, 10), 4.19 (1H, br m, H-7), 3.67 (3H, s, CO_2Me) , 3.37 (1H, dd, *J* = 13.8, 6.0 Hz, H-11a), 3.16 (1H, dd, *J* = 7.2, 7.2 Hz, H-23), 2.81 (1H, dd, *J* = 13.2, 4.8 Hz, H-29a), 2.72 (1H, dd, *J* = 13.2, 9.6 Hz, H-29b), 2.71-2.65 (2H, overlapped, H-24), 2.44 (1H, dd, *J* = 13.8, 11.4 Hz, H-11b), 2.14 (6H, s, H-35); ^{13}C{^{1}H} NMR (150 MHz, $CDCl_3$) δ 172.9 (C-22), 170.8 (C-34), 169.5 (C-8), 166.6 (C-5), 159.0 (C-1), 157.0 (C-28), 137.9 (C-17), 137.2 (Bn), 135.8 (C-30), 132.1 (C-25), 131.6, 131.2, 130.8, 130.0 (C-26), 129.2 (C-31), 128.6, 128.5, 128.4, 128.0, 127.9, 127.5, 126.9, 125.8 (C-18), 121.5, 119.4, 114.6, 86.2 (C-3), 71.3 (C-23), 70.0 (Bn), 56.4 (C-4), 54.9 (C-7), 52.9 (C-10), 52.5 (Me), 42.5 (C-35), 39.9 (C-29), 38.3 (C-11), 33.1 (C-24); HRMS (ESI) m/z: $[M+H]^+$ calcd for $C_{46}H_{49}N_4O_7^+$ 769.3601, found 769.3581; IR (ATR) ν_{max} 3308, 3032, 2931, 1739, 1648, 1509, 1226, 1029, 751, 698 cm^{-1}.

*Carboxylic acid **27***. To a solution of **2** (2.5 mg, 3.3 μmol) in THF (0.15 mL) was added 0.066 N LiOH aq. (0.15 mL, 3 eq.) at 0 °C under Ar atmosphere. The reaction mixture was warmed to room temperature and stirred for 15 hours at the same temperature. The reaction was quenched by adding AcOH to pH 3–4. The aqueous layer was extracted three times with $CHCl_3$. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was purified by silica gel preparative thin-layer chromatography (MeOH/$CHCl_3$ = 1:4) to afford carboxylic acid **27** as a white solid (2.4 mg, 98%). Otherwise, the residue was used in the next reaction without purification. $[\alpha]^{27}_{D}$ +18.8 (*c* 0.12, MeOH);.^{1}H NMR (600 MHz, CD_3OD) δ 7.58 (2H, d, *J* = 7.6 Hz), 7.41 (2H, d, *J* = 6.9 H), 7.37–7.36 (4H, overlapped), 7.31–7.26 (2H, overlapped), 7.17–7.14 (5H, overlapped), 7.05 (1H, m), 6.88 (1H, dd, *J* = 8.2, 2.1 Hz), 6.85 (1H, dd, *J* = 8.2, 2.1 Hz), 6.81-6.79 (3H, overlapped), 6.72-6.71 (2H, overlapped), 5.75 (1H, s), 5.00 (2H, s), 4.85 (1H, d, *J* = 2.1 Hz), 4.68 (1H, dd, *J* = 11.7, 6.2 Hz), 4.20 (1H, dd, *J* = 9.0, 4.7 Hz), 3.45 (1H, dd, *J* = 13.1, 6.2 Hz), 3.14 (1H, m), 2.92 (1H, dd, *J* = 13.7, 4.7 Hz), 2.72 (1H, dd, *J* = 13.7, 9.0 Hz), 2.68-2.61 (2H, overlapped), 2.53 (1H, dd, *J* = 12.4, 12.4 Hz), 1.96 (6H, s); ^{13}C{^{1}H} NMR (150 MHz, CD_3OD) δ 178.4, 173.3, 171.4, 168.6, 159.6, 158.6, 140.5, 139.0, 138.6, 135.1, 132.5, 132.2, 131.5, 131.1, 130.5, 129.5, 129.3, 128.8, 128.6, 128.5, 127.5, 127.1, 122.6, 120.1, 116.8, 87.0, 71.7, 71.0, 57.7, 57.4, 55.7, 42.5, 40.1, 40.1, 35.6; HRMS (ESI) m/z: $[M+H]^+$ calcd for $C_{45}H_{47}N_4O_7^+$ 755.3445, found 755.3449; IR (ATR) ν_{max} 3310, 3032, 2925, 1652, 1610, 1512, 1454, 1376, 1227, 1177, 1028, 748, 698 cm^{-1}.

*Zwitterionic Compound **28***. To a solution of crude **27** (9.2 mg, <9.6 μmol) in MeOH/acetonitrile (1:5,

0.5 mL, 0.02 M) were added AcOH (2.7 μL, 5 eq.), DIPEA (6.7 μL, 4 eq.), and MeI (6.1 μL, 10 eq.) at room temperature under Ar atmosphere. After stirring for 2 hours at the same temperature, the reaction mixture was concentrated under reduced pressure. The residue was purified by ODS flash chromatography (H_2O/MeOH = 1:3) to afford zwitterionic compound **28** as a white solid (4.3 mg, 58%), together with carboxylic acid **27** (1.8 mg, 24%). $[\alpha]^{23}_D$ +6.0 (*c* 0.22, MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 7.62 (2H, d, *J* = 7.6 Hz), 7.43 (2H, d, *J* = 7.6 Hz), 7.39–7.38 (4H, overlapped), 7.32 (1H, t, *J* = 7.6 Hz), 7.29 (1H, t, *J* = 7.6 Hz), 7.22–7.21 (4H, overlapped), 7.16 (1H, dd, *J* = 8.2, 2.1 Hz), 7.11 (1H, m), 6.85 (1H, dd, *J* = 8.2, 2.1 Hz), 6.82-6.81 (2H, overlapped), 6.78 (1H, dd, *J* = 8.2, 2.4 Hz), 6.75 (1H, dd, *J* = 8.2, 2.4 Hz), 6.64–6.62 (2H, overlapped), 5.77 (1H, d, *J* = 2.1 Hz), 5.11 (1H, d, *J* = 2.1 Hz), 5.00 (1H, d, *J* = 11.7 Hz), 4.97 (1H, d, *J* = 11.7 Hz), 4.68 (1H, dd, *J* = 11.7, 6.0 Hz), 4.15 (1H, dd, *J* = 9.6, 4.1 Hz), 3.92 (1H, dd, *J* = 10.7, 3.4 Hz), 3.45 (1H, dd, *J* = 13.1, 6.0 Hz), 3.11 (1H, dd, *J* = 13.1, 3.4 Hz), 3.06 (1H, dd, *J* = 13.1, 10.7 Hz), 2.94 (1H, dd, *J* = 13.7, 4.1 Hz), 2.71 (9H, s), 2.65 (1H, dd, *J* = 13.7, 9.6 Hz), 2.52 (1H, dd, *J* = 13.1, 11.7 Hz); ^{13}C{^{1}H} NMR (150 MHz, CD_3OD) δ 178.3, 171.4, 166.8, 166.6, 159.5, 159.4, 140.5, 139.0, 138.7, 135.1, 132.6, 131.6, 131.5, 130.6, 129.5, 129.4, 128.9, 128.9, 128.5, 127.6, 127.1, 126.7, 122.4, 119.7, 116.5, 86.6, 77.0, 71.0, 57.8, 57.3, 55.6, 52.5, 40.2, 40.1, 32.9; HRMS (ESI) m/z: $[M+H]^+$ calcd for $C_{46}H_{49}N_4O_7^+$ 769.3601, found 769.3620; IR (ATR) ν_{max} 3298, 3064, 3036, 2925, 1644, 1609, 1541, 1515, 1455, 1392, 1233, 1178, 1038, 740, 701 cm^{-1}.

*Ophiorrhisine A (**1**)*. To a solution of **28** (3.4 mg, 4.4 μmol) in MeOH (0.2 mL, 0.02 M) was added Pd/C (10%, 1.4 mg, 30 mol%) at room temperature. The reaction mixture was stirred for 1.5 h at room temperature under hydrogen atmosphere. The reaction mixture was filtered through a pad of Celite® with MeOH. The filtrate was evaporated under reduced pressure. The residue was purified by ODS flash chromatography (H_2O/MeOH = 1:1) to afford ophiorrhisine A (**1**) as a colorless solid (2.7 mg, 90%): $[\alpha]^{24}_D$ +5.7 (*c* 0.13, MeOH); ECD (*c* 0.15 mM, MeOH, 24 °C, Synthetic) $\Delta\varepsilon$ (λ nm) +0.4 (258), +27.4 (233), +15.6 (226), –0.2 (217), –12.6 (212), 0 (207); [ECD (*c* 0.17 mM, MeOH, 24 °C, Natural) $\Delta\varepsilon$ (λ nm) +0.4 (258), +11.2 (234), +7.1 (227), –0.3 (218), –5.0 (213), –2.0 (207)]; ^{1}H NMR (600 MHz, CD_3OD) δ 7.61 (2H, d, *J* = 7.6 Hz, H-18), 7.37 (2H, d, *J* = 7.6 Hz, H-19), 7.29 (1H, t, *J* = 7.2 Hz, H-20), 7.24 (2H, dd, *J* = 8.2, 7.6 Hz, H-32), 7.20 (2H, d, *J* = 8.2 Hz, H-31), 7.15 (1H, dd, *J* = 8.2, 2.1 Hz, H-13), 7.11 (1H, t, *J* = 7.6 Hz, H-33), 6.87 (1H, dd, *J* = 8.2, 2.1 Hz, H-16), 6.78 (1H, dd, *J* = 8.2, 2.1 Hz, H-15), 6.77 (2H, d, *J* = 8.2 Hz, H-26), 6.75 (1H, dd, *J* = 8.2, 2.4 Hz, H-14), 6.58 (2H, d, *J* = 8.2 Hz, H-27), 5.78 (1H, d, *J* = 2.1 Hz, H-3), 5.04 (1H, d, *J* = 2.1 Hz, H-4), 4.67 (1H, dd, *J* = 11.7, 6.2 Hz, H-10), 4.18 (1H, dd, *J* = 9.6, 4.8 Hz, H-7), 3.95 (1H, m, H-23), 3.45 (1H, dd, *J* = 13.1, 6.2 Hz, H-11a), 3.08 (1H, dd, *J* = 13.7, 3.4 Hz, H-24a), 3.03 (1H, dd, *J* = 13.1, 9.6 Hz, H-24b), 2.94 (1H, dd, *J* = 13.7, 4.8 Hz, H-29a), 2.70 (9H, s, H-35), 2.66 (1H, dd, *J* = 13.7, 9.6 Hz, H-29b), 2.53 (1H, dd, *J* = 13.1, 11.7 Hz, H-11b); ^{13}C{^{1}H} NMR (150 MHz, CD_3OD) δ 178.4, 171.3, 167.0, 166.7, 159.3, 158.0, 140.5, 138.7, 135.1, 132.6, 131.5, 130.5, 129.4, 128.8, 127.6, 127.1, 125.4, 122.5, 119.7, 117.0, 86.4, 77.1, 57.9, 57.4, 55.5, 52.6, 40.1,

33.1; HRMS (ESI) m/z: $[M+H]^+$ calcd for $C_{39}H_{43}N_4O_7^+$ 679.3132, found 679.3119; IR (ATR) ν_{max} 3396, 3057, 1660, 1592, 1516, 1452, 1395, 1224 cm^{-1}.

*Derivative **29***. To a solution of **3** (7.2 mg, 11 μmol) and Pd/C (10%, 3.4 mg, 30 mol%) in MeOH/DCM (1:1, 0.54 mL, 0.02 M) was added formalin (7.5 μL, 9 eq.) at room temperature. The reaction mixture was stirred for 18 hours at room temperature under hydrogen atmosphere. The reaction mixture was filtered through a pad of Celite® with MeOH. The filtrate was evaporated under reduced pressure. The residue was purified by amino silica gel flash chromatography (AcOEt/hexane = 3:2 to 4:1 gradient) to afford derivative **29** as a white solid (3.3 mg, 59%): $[\alpha]^{22}_D$ –32.3 (*c* 0.17, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$) δ 7.51 (2H, d, *J* = 8.2 Hz), 7.41 (2H, dd, *J* = 7.9, 7.9 Hz), 7.33 (1H, dd, *J* = 7.6, 7.6 Hz), 7.25 (2H, dd, *J* = 7.9, 7.9 Hz), 7.20 (1H, dd, *J* = 7.6, 7.6 Hz), 7.13-7.11 (3H, overlapped), 7.04 (1H, dd, *J* = 8.2, 2.1 Hz), 6.84-6.83 (2H, overlapped), 5.76 (1H, d, *J* = 8.6 Hz, NH), 5.55 (1H, d, *J* = 3.6 Hz), 5.15 (1H, d, *J* = 10.3 Hz), 4.92 (1H, ddd, *J* = 11.3, 11.3, 6.0 Hz), 4.21 (1H, ddd, *J* = 9.0, 7.8, 7.2 Hz), 3.74 (3H, s), 3.41 (1H, dd, *J* = 13.2, 6.0 Hz), 3.36 (1H, d, *J* = 3.6 Hz), 2.78 (2H, d, *J* = 7.6 Hz), 2.49 (1H, dd, *J* = 13.2, 12.0 Hz), 2.45 (6H, s, NMe_2); $^{13}C\{^1H\}$ NMR (150 MHz, $CDCl_3$) δ 171.2, 170.0, 168.9, 160.0, 139.5, 136.2, 131.4, 130.6, 130.5, 129.3, 128.5, 128.2, 127.3, 126.8, 125.6, 121.6, 118.9, 87.9, 69.8, 54.0, 53.0, 52.5, 42.6, 40.0, 38.4; HRMS (ESI) m/z: $[M+H]^+$ calcd for $C_{30}H_{34}N_3O_5^+$ 516.2499, found 516.2501; IR (ATR) ν_{max} 3318, 3063, 3030, 2952, 2926, 2882, 1739, 1653, 1511, 1447, 1368, 1281, 1236, 1170, 1115, 1023 cm^{-1}.

*Derivative **30***. To a solution of **26** (11.7 mg, 17.5 μmol) in MeOH/DCM (1:1, 0.7 mL, 0.025 M) was added Pd/C (10%, 5.6 mg, 30 mol%) at room temperature. The reaction mixture was stirred for 3.5 hours at room temperature under hydrogen atmosphere. The reaction mixture was filtered through a pad of Celite® with MeOH. The filtrate was evaporated under reduced pressure and the residue was used in the next reaction without further purification. To a solution of the above residue (8.7 mg) in DCM (0.7 mL, 0.03 M) was added Ac_2O (3.3 μL, 2 eq.) at 0 °C under Ar atmosphere. After the addition, the reaction mixture was allowed to warm to room temperature and stirred for 14.5 hours. The reaction mixture was diluted with $CHCl_3$ and washed with saturated $NaHCO_3$ aq., 10% citric acid aq., and brine. The organic layer was dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was purified by silica gel flash chromatography (AcOEt) to afford derivative **30** as a white solid (7.5 mg, 80% over 2 steps): $[\alpha]^{22}_D$ –15.6 (*c* 0.37, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$) δ 7.47 (2H, d, *J* = 7.6 Hz), 7.40 (2H, dd, *J* = 7.6, 7.6 Hz), 7.33 (1H, dd, *J* = 7.6, 7.6 Hz), 7.24-7.22 (3H, overlapped), 7.15 (1H, dd, *J* = 8.2, 2.1 Hz), 7.07 (2H, overlapped), 6.94 (1H, dd, *J* = 8.4, 2.4 Hz), 6.88 (1H, dd, *J* = 8.6, 2.4 Hz), 6.81 (1H, dd, *J* = 8.2, 2.1 Hz), 6.42 (1H, d, *J* = 9.6 Hz, NH), 6.15 (1H, d, *J* = 8.9 Hz, NH), 5.56 (1H, d, *J* = 2.1 Hz), 5.06 (1H, d, *J* = 10.3 Hz), 4.99 (1H, dd, *J* = 9.6, 2.1 Hz), 4.87 (1H, ddd, *J* = 11.2, 11.2, 5.7 Hz), 4.25 (1H, ddd, *J* = 9.3, 9.3, 5.5 Hz, H-7), 3.69 (3H, s) , 3.37 (1H, dd, *J* = 13.4, 6.2 Hz), 2.81 (1H, dd, *J* = 13.4, 5.2 Hz), 2.77 (1H, dd, *J* = 13.4, 9.6 Hz), 2.44 (1H, dd, *J* = 13.4, 12.0 Hz), 1.91 (3H, s, Ac); $^{13}C\{^1H\}$ NMR (150 MHz, $CDCl_3$) δ 170.9, 170.0, 169.5, 166.9, 159.2, 137.7, 135.6, 131.7, 131.0, 130.8, 129.3, 128.5, 128.4,

128.1, 127.0, 126.0, 121.1, 119.0, 86.8, 56.1, 54.7, 52.9, 52.4, 40.2, 38.3, 22.9; HRMS (ESI) m/z: $[M+Na]^+$ calcd for $C_{30}H_{31}N_3NaO_6^+$ 552.2111, found 552.2125; IR (ATR) ν_{max} 3308, 3063, 3032, 2952, 2927, 1740, 1644, 1509, 1452, 1382, 1280, 1227, 1170, 1032 cm^{-1}.

*Derivative **31***. To a solution of **2** (6.4 mg, 8 μmol) in MeOH/DCM (1:1, 0.2 mL, 0.04 M) was added $Pd(OH)_2/C$ (20%, 2.8 mg, 50 mol%) at room temperature. The reaction mixture was stirred for 1.5 hours at room temperature under hydrogen atmosphere. The reaction mixture was filtered through a pad of Celite® with MeOH. The filtrate was evaporated under reduced pressure. The residue was purified by silica gel preparative thin-layer chromatography ($MeOH/CHCl_3$ = 1:19) to afford derivative **31** as a white solid (3.5 mg, 62%): $[\alpha]^{20}_D$ +13.8 (*c* 0.18, $CHCl_3$); ^1H NMR (600 MHz, CD_3OD) δ 7.59 (2H, d, *J* = 8.4 Hz), 7.39 (2H, dd, *J* = 8.4, 8.4 Hz), 7.29 (1H, dd, *J* = 8.4, 8.4 Hz), 7.21 (2H, dd, *J* = 7.5, 7.5 Hz), 7.15–7.12 (4H, overlapped), 6.89 (1H, dd, *J* = 8.1, 2.1 Hz), 6.87 (1H, dd, *J* = 8.1, 2.1 Hz), 6.83 (1H, dd, *J* = 8.4, 2.1 Hz), 6.81 (2H, d, *J* = 8.4 Hz), 6.61 (2H, d, *J* = 8.4 Hz), 5.76 (1H, s), 4.91 (1H, s), 4.85 (1H, d, *J* = 6.0 Hz), 4.83 (1H, dd, *J* = 12.0, 6.6 Hz), 4.17 (1H, dd, *J* = 8.4, 7.2 Hz), 3.67 (3H, s), 3.36–3.31 (2H, overlapped), 2.81 (1H, dd, *J* = 13.2, 8.4 Hz), 2.76 (2H, d, *J* = 6.6 Hz), 2.67 (1H, dd, *J* = 13.2, 6.6 Hz), 2.56 (1H, dd, *J* = 13.2, 12.6 Hz), 2.10 (6H, s, NMe_2); $^{13}C\{^1H\}$ NMR (150 MHz, CD_3OD) δ 172.7, 171.7, 168.3, 160.0, 157.1, 140.3, 137.9, 133.4, 132.8, 131.2, 130.4, 129.4, 129.4, 128.7, 127.6, 127.0, 122.7, 120.3, 116.2, 86.9, 71.4, 58.0, 55.5, 54.2, 52.7, 42.4, 40.7, 38.2, 35.5; HRMS (ESI) m/z: $[M+H]^+$ calcd for $C_{39}H_{43}N_4O_7^+$ 679.3132, found 679.3117; IR (ATR) ν_{max} 3321, 3061, 3029, 2954, 1734, 1653, 1614. 1516, 1450, 1376, 1225, 1172, 1039, 825, 759 cm^{-1}.

*Derivative **32***. To a solution of **3** (7.4 mg, 0.015 mmol) in MeOH/DCM (1:1, 0.3 mL, 0.05 M) were added *N*-Cbz-L-phenylalanine (6.8 mg, 1.5 eq.), DIPEA (10.5 μL, 4 eq.), and DMT-MM (8.3 mg, 2 eq.) at room temperature. After stirring for 4 hours at the same temperature under Ar atmosphere, the reaction was quenched by adding 10% citric acid aq. The aqueous layer was extracted three times with AcOEt. The combined organic layers were washed with saturated $NaHCO_3$ aq. and brine, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was purified by silica gel flash chromatography (AcOEt/hexane = 1:1 to 7:3 gradient) to afford corresponding tetrapeptide as a white solid (10.4 mg, 89%): $[\alpha]^{21}_D$ +2.4 (*c* 0.32, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$) δ 7.46 (2H, d, *J* = 7.6 Hz), 7.35–7.15 (18H, overlapped), 6.91 (2H, br s), 6.82 (2H, overlapped), 6.21 (1H, s), 5.62 (1H, s), 5.18 (1H, d, *J* = 8.2 Hz), 5.10 (1H, d, *J* = 12.4 Hz), 5.02 (1H, d, *J* = 12.4 Hz), 4.99 (1H, d, *J* = 8.9 Hz), 4.85 (1H, ddd, *J* = 11.0, 11.0, 6.2 Hz), 4.47 (1H, br s), 3.65 (3H, s), 3.32 (1H, dd, *J* = 13.2, 5.8 Hz), 3.00 (1H, dd, *J* = 14.2, 5.8 Hz), 2.91 (1H, br s), 2.80 (1H, dd, *J* = 13.1, 5.5 Hz), 2.75 (1H, dd, *J* = 13.1, 9.6 Hz), 2.43 (1H, dd, *J* = 12.4, 12.4 Hz); $^{13}C\{^1H\}$ NMR (150 MHz, $CDCl_3$) δ 171.2, 171.0, 169.6, 166.5, 159.3, 155.9, 137.6, 136.6, 136.6, 135.8, 132.0, 131.1, 130.9, 129.6, 129.4, 128.6, 128.4, 128.2, 128.2, 128.1, 127.9, 127.0, 126.9, 126.4, 121.5, 119.1, 86.8, 67.0, 57.0, 54.5, 53.0, 52.2, 40.2, 39.0, 38.4; HRMS (ESI) m/z: $[M+Na]^+$ calcd for $C_{45}H_{44}N_4NaO_8^+$ 791.3057, found 791.3076; IR (ATR) ν_{max} 3313, 3064, 3031, 2961,

2924, 2853, 1732, 1706, 1686, 1644, 1509, 1454, 1387, 1348, 1261, 1219, 1169, 1096, 1028, 804, 744, 699 cm^{-1}. To a solution of corresponding tetrapeptide (9.5 mg, 12 μmol) and Pd/C (10%, 14.2 mg, 110 mol%) in MeOH (0.6 mL, 0.02 M) was added formalin (13.8 μL, 15 eq.) at room temperature. The reaction mixture was stirred for 8.5 hours at room temperature under hydrogen atmosphere. The reaction mixture was filtered through a pad of Celite® with MeOH. The filtrate was evaporated under reduced pressure. The residue was purified by silica gel flash chromatography (MeOH/$CHCl_3$ = 1:19) to afford derivative **32** as a white solid (3.3 mg, 40%): $[\alpha]^{23}_D$ +4.0 (*c* 0.11, $CHCl_3$); ^1H NMR (600 MHz, CD_3OD) δ 7.60 (2H, d, *J* = 7.6 Hz), 7.40 (2H, d, *J* = 7.6 Hz), 7.30 (1H, dd, *J* = 7.6, 7.6 Hz), 7.24 (2H, dd, *J* = 7.2, 7.2 Hz), 7.16–7.12 (7H, overlapped), 6.98 (2H, d, *J* = 6.9 Hz), 6.90-6.86 (2H, overlapped), 6.84 (1H, dd, *J* = 8.2, 2.1 Hz), 5.77 (1H, s), 4.85–4.83 (2H, overlapped), 4.18 (1H, dd, *J* = 7.6, 7.6 Hz), 3.67 (3H, s), 3.35–3.32 (2H, overlapped), 2.83–2.80 (3H, overlapped), 2.66 (1H, dd, *J* = 13.4, 7.2 Hz), 2.65 (1H, dd, *J* = 12.7, 12.7 Hz), 2.05 (6H, s); $^{13}C\{^1H\}$ NMR (150 MHz, CD_3OD) δ 172.7, 171.7, 168.5, 160.0, 140.3, 137.9, 133.4, 132.7, 131.3, 130.4, 130.2, 129.4, 128.7, 127.6, 127.3, 127.0, 122.8, 120.3, 86.9, 71.3, 58.0, 55.5, 54.2, 52.7, 42.4, 40.7, 38.2, 36.2; HRMS (ESI) m/z: $[M+H]^+$ calcd for $C_{39}H_{43}N_4O_6^+$ 663.3183, found 663.3182; IR (ATR) ν_{max} 3318, 3029, 2950, 2930, 1742, 1643, 1607, 1507, 1454, 1435, 1363, 1282, 1225, 1171, 1033 cm^{-1}.

*N,N-Dimethyltryptophan•HCl salt (**33**)*. To a solution of *N*-Cbz-L-tryptophan (338.4 mg, 1.0 mmol), BnOH (154 mL, 1.5 eq.), and DMAP (24 mg, 20 mol%) in DCM (2 mL, 0.5 M) was added DCC (206.3 mg, 1.0 eq.) at room temperature. After stirring for 4 hours at the same temperature under Ar atmosphere, the reaction mixture was passed through a silica gel pad with AcOEt to afford a crude residue. The residue was used in the next reaction without further purification. To a solution of the above residue in THF (5 mL, 0.2 M) was added Boc_2O (218 μL, 1.0 eq.) in the presence of DMAP (24 mg, 20 mol%) at room temperature. More Boc_2O was added until the reaction was completed. The reaction mixture was quenched by adding 10% citric acid aq. and extracted once with AcOEt. The organic layer was washed with saturated $NaHCO_3$ aq. and brine, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was purified by silica gel flash chromatography (AcOEt/hexane = 1:4) to afford *N*-Cbz-L-Trp(Boc)OBn as a colorless oil (477.5 mg, 90%): $[\alpha]^{22}_D$ +18.4 (*c* 1.31, $CHCl_3$); UV (MeOH) λ_{max} (nm): 294, 285, 264, 259, 229, 203; ^1H NMR (600 MHz, $CDCl_3$) δ 8.11 (1H, br s), 7.46 (1H, d, *J* = 7.6 Hz), 7.34–7.32 (10H, overlapped), 7.19–7.17 (3H, overlapped), 5.38 (1H, d, *J* = 7.6 Hz), 5.13–5.08 (3H, overlapped), 5.04 (1H, d, *J* = 11.7 Hz), 4.78 (1H, dd, *J* = 13.7, 6.2 Hz), 3.26-3.22 (2H, m), 1.64 (9H, s); $^{13}C\{^1H\}$ NMR (150 MHz, $CDCl_3$) δ 171.4, 155.6, 149.5, 136.2, 135.3, 134.9, 130.4, 128.6, 128.5, 128.4, 128.2, 128.1, 124.5, 124.2, 122.6, 118.9, 115.3, 114.7, 83.7, 67.4, 67.0, 54.2, 28.2, 28.0; HRMS (ESI) m/z: $[M+Na]^+$ calcd for $C_{31}H_{32}N_2NaO_6^+$ 551.2158, found 551.2186; IR (ATR) ν_{max} 3361, 2978, 1732, 1519, 1455, 1372, 1257, 1159, 1088, 1060, 749, 698 cm^{-1}. To a solution of *N*-Cbz-L-Trp(Boc)OBn

(8.8 mg, 0.017 mmol) and formalin (5 μL, 4 eq.) in AcOEt/MeOH (2:1, 0.2 mL, 0.1 M) was added Pd/C (1.8 mg, 10 mol%) at room temperature. After stirring for 3 hours under hydrogen atmosphere, the reaction mixture was filtered through a pad of Celite® with MeOH. The filtrate was used in the next reaction without further purification. To a solution of the above filtrate was added 4 N HCl aq. (1 mL) at room temperature. After stirring for 1.5 hours at the same temperature, the reaction mixture was evaporated with a base trap under reduced pressure. The residue was purified by ODS chromatography (H_2O/MeOH = 3:2) to afford *N*,*N*-dimethyltryptophan•HCl salt (**33**) as a pale brown solid (4.2 mg, 96% over 2 steps): $[\alpha]^{23}_D$ +57.6 (*c* 0.21, 0.2 N HCl aq.); UV (MeOH) λ_{max} (nm): 290, 281, 274, 220; ^{1}H NMR (600 MHz, D_2O) δ 7.58 (1H, d, *J* = 8.2 Hz), 7.39 (1H, d, *J* = 8.2 Hz), 7.19 (1H, s), 7.14 (1H, dd, *J* = 8.2, 6.9 Hz), 7.07 (1H, dd, *J* = 8.2, 6.9 Hz), 3.82 (1H, dd, *J* = 7.6, 6.2 Hz), 3.35 (1H, dd, *J* = 15.5, 6.2 Hz), 3.28 (1H, dd, *J* = 15.5, 7.6 Hz), 2.83 (3H, s), 2.75 (3H, s); ^{13}C{^{1}H} NMR (150 MHz, D_2O) δ 173.4, 136.7, 127.2, 125.2, 122.7, 120.0, 119.0, 112.6, 108.1, 71.4, 43.3, 41.0, 24.6; HRMS (ESI) m/z: $[M+H]^+$ calcd for $C_{13}H_{17}N_2O_2^+$ 233.1290, found 233.1268; IR (ATR) ν_{max} 3406, 3260, 3057, 2925, 1625, 1458, 1393, 1357, 748 cm^{-1}.

*Derivative **34***. To a solution of **3** (9.0 mg, 18 μmol), **33** (5.1 mg, 1.2 eq.), and DIPEA (12.6 μL, 4 eq.) in MeOH/MeCN (1:1, 0.36 mL, 0.05 M) was added DMT-MM (10.0 mg, 2 eq.) at room temperature. After stirring for 4 hours, the reaction mixture was quenched by adding 10% citric acid aq. and extracted three times with AcOEt. The combined organic layers were washed with saturated $NaHCO_3$ aq. and brine, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was purified by silica gel preparative thin-layer chromatography (MeOH/$CHCl_3$ = 1:19) to afford derivative **34** as a colorless solid (6.7 mg, 52%): $[\alpha]^{21}_D$ +33.8 (*c* 0.30, MeOH); UV (MeOH) λ_{max} (nm): 290, 282, 273, 219; ^{1}H NMR (600 MHz, CD_3OD) δ 7.56 (2H, d, *J* = 7.6 Hz), 7.52 (1H, d, *J* = 8.2 Hz), 7.36 (2H, dd, *J* = 7.6, 7.6 Hz), 7.32 (1H, d, *J* = 8.2 Hz), 7.27 (1H, dd, *J* = 7.2, 7.2 Hz), 7.21–7.19 (2H, overlapped), 7.13-7.11 (4H, overlapped), 7.07 (1H, dd, *J* = 7.6, 7.6 Hz), 6.99 (1H, dd, *J* = 7.6, 7.6 Hz), 6.89–6.86 (2H, overlapped), 6.82 (1H, dd, *J* = 8.2, 2.1 Hz), 6.79 (1H, s), 5.76 (1H, s, H-3), 4.88 (1H, overlapped with DOH), 4.83 (1H, dd, *J* = 11.7, 6.2 Hz), 4.18 (1H, dd, *J* = 7.6, 7.6 Hz), 3.66 (3H, s), 3.55 (1H, m), 3.33 (1H, m), 3.05 (2H, d, *J* = 6.9 Hz), 2.81 (1H, dd, *J* = 13.1, 8.2 Hz), 2.66 (1H, dd, *J* = 13.1, 6.9 Hz), 2.56 (1H, dd, *J* = 12.4, 12.4 Hz), 2.15 (6H, s); ^{13}C{^{1}H} NMR (150 MHz, CD_3OD) δ 172.7, 171.7, 168.4, 160.0, 140.3, 138.0, 137.9, 133.4, 132.7, 131.3, 130.5, 129.4, 129.3, 128.7, 128.6, 127.6, 127.0, 124.7, 122.7, 122.3, 120.3, 119.7, 119.1, 112.3, 87.0, 70.0, 58.0, 55.5, 54.3, 52.7, 42.4, 40.7, 38.2, 26.0; HRMS (ESI) m/z: $[M+H]^+$ calcd for $C_{41}H_{44}N_5O_6^+$ 702.3292, found 702.3305; IR (ATR) ν_{max} 3312, 1741, 1706, 1652, 1609, 1508, 1455, 1437, 1362, 1281, 1225, 1171, 1032, 743, 701 cm^{-1}.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI:

^{1}H and $^{13}C\{^{1}H\}$ NMR spectra for synthetic compounds (PDF)

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Notes

The authors declare no competing financial interest.

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