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Total Synthesis of Miuraenamides A and D

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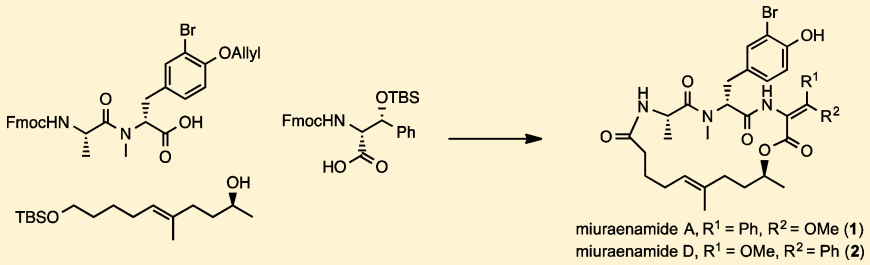
Supporting Information



ABSTRACT: Miuraenamides A and D, cyclodepsipeptides with antimicrobial and antitumor activity, were synthesized. The synthesis of an unsaturated hydroxycarboxylic acid moiety, starting from a chiral epoxide, was achieved by Suzuki−Miyaura coupling as a key step. As a result, the overall yield for miuraenamide A over the longest linear sequence is 3.2%, while the yield of the previously reported procedure is 1.9%. In addition, the cell growth-inhibitory activity and anti-*Phytophthora* activity of the synthesized compounds were evaluated.

INTRODUCTION

Miuraenamide A (**1**) is a cyclic hybrid polyketide-peptide antibiotic that was isolated from *Paraliomixa miuraensis*, a slightly halophilic myxobacterium discovered at the seashore on Miura Peninsula in Kanagawa, Japan by Ojika et al. in 2006 (Figure 1).[1] Its absolute stereochemistry was determined in 2008.[2] Miuraenamide A (**1**) shows antimicrobial activity,[1,2] inhibits NADH oxidase,[1] and stabilizes actin filaments.[3] The β-methoxyacrylate group of **1** is known to be important for its antimicrobial activity.[2] On the other hand, its geometrical isomer, miuraenamide D (**2**) (Figure 1), shows weaker antimicrobial activity than **1**. Compounds that are structurely related to **1** have also been isolated, such as jasplakinolides,[4] chondramides,[5] geodiamolide,[6] and doliculide,[7] which are macrocyclic depsipeptides that contain peptide and polyketide moieties, and were found to interact with actin and induce apoptosis.

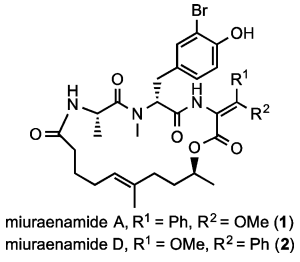
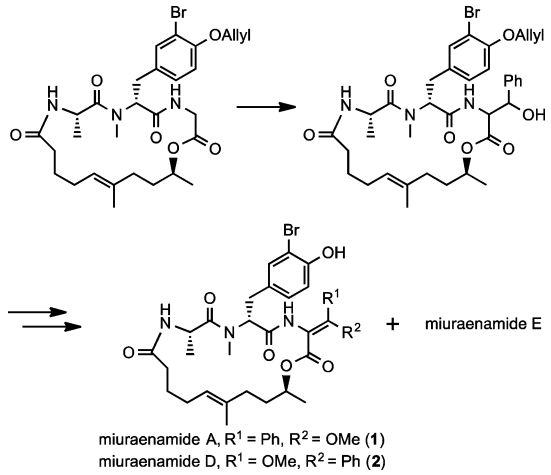


Figure 1. Structure of miuraenamides A (**1**) and D (**2**).

In 2015, Kazmaier et al. achieved the total synthesis of miuraenamides A (**1**), D (**2**), and E (Scheme 1).[8] Their synthetic method should allow the synthesis of miuraenamide derivatives for studies of structure−activity relationships and

Scheme 1. Kazmaier's Synthetic Strategy



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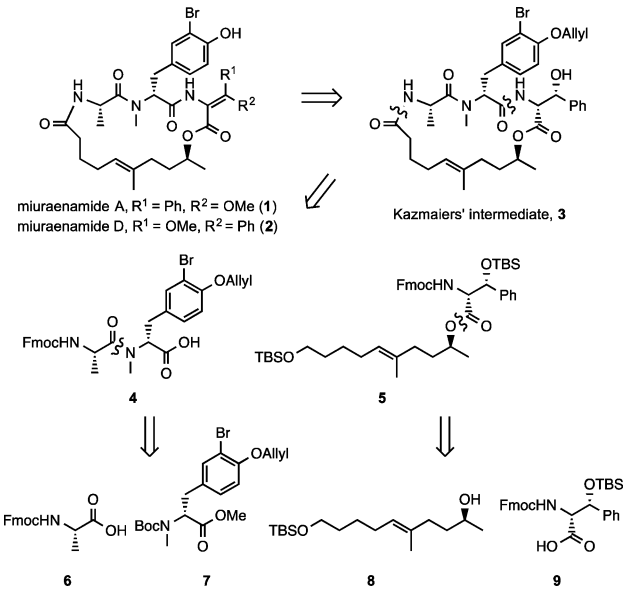
indeed they investigated the structure−activity relationships of miuraenamides by evaluating the cytotoxicity of synthetic miuraenamides A (**1**), D (**2**), and E, as well as their synthetic precursors and analogues against human cancer cells.[8] They introduced an unusual enamine side chain in the final stage of their synthesis (Scheme 1). On the other hand, we introduced the required functional group in the early stage of our synthesis.

We envisioned that miuraenamides A (**1**) and D (**2**) could be synthesized via Kazmaier's intermediate **3** (Scheme 2). It could

Scheme 2. Retrosynthesis of Miuraenamides A and D



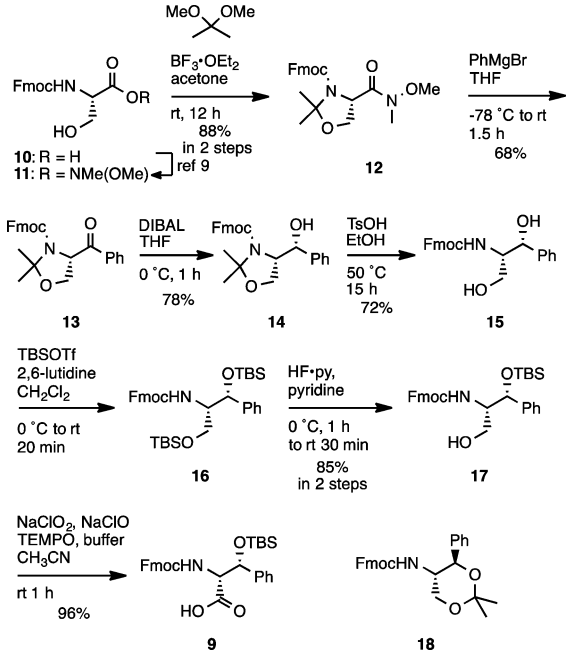
be accessed from dipeptide **4** and ester **5** through several reactions including their condensation and macrolactamization. Dipeptide **4** and ester **5** could be obtained from the building blocks **6**, **7**, **8**, and **9**, as shown in Scheme 2.

RESULTS AND DISCUSSION

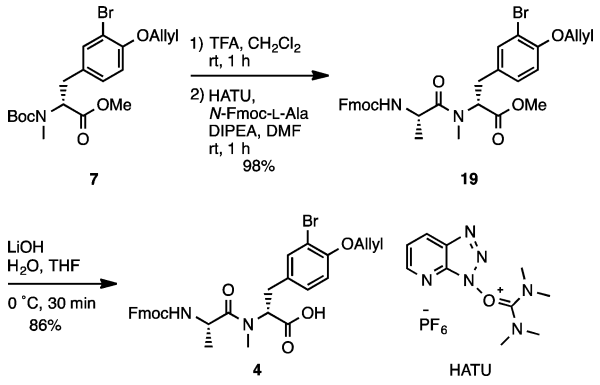
Our synthesis began with the preparation of phenylserine derivative **9** (Scheme 3). *N*-Fmoc-L-serine (**10**) was converted to the Weinreb amide **11** by a known procedure without undesired condensation between the hydroxy group and carboxylic acid.[9] The protection of **11** with 2,2-dimethoxypropane gave rise to acetonide **12**. Subsequent Grignard reaction using PhMgBr afforded ketone **13**. Chelation-controlled stereoselective reduction of **13** was achieved with DIBAL to provide alcohol **14** as a single diastereomer.[10] The stereochemistry was determined based on ^{1}H NMR spectra, and the results of NOESY experiments after conversion into the derived 6-membered acetal **18** (For details, see the Supporting Information, SI, p 2).[11] Removal of the acetonide group and protection of the two hydroxy groups as TBS ethers with TBSOTf, followed by selective deprotection of the primary TBS ether, afforded alcohol **17**. Finally, oxidation of the hydroxy group to a carboxyl group provided the phenylserine derivative **9** in good yield.[12]

Synthesis of the dipeptide **4** started from the known bromotyrosine derivative **7**[8] (Scheme 4). Removal of the Boc protecting group and condensation with *N*-Fmoc-L-alanine using HATU[13] as a condensation reagent followed by saponification generated the desired dipeptide **4**.

Scheme 3. Synthesis of Phenylserine Derivative 9



Scheme 4. Synthesis of Dipeptide 4



Suzuki−Miyaura coupling was chosen as the key step in the synthesis of the required unsaturated alcohol **8** (Scheme 5). Starting from (*S*)-(−)-propylene oxide (**20**), Grignard reaction with propargyl bromide catalyzed by $HgCl_2$ provided known alkyne **21**.[14] Subsequent methyl alumination followed by iodination gave rise to vinyl iodide **22**. After the hydroxy group was protected as an acetate, Suzuki−Miyaura coupling with commercially available iodide **24**, followed by reductive removal of the acetyl group, provided the required known alcohol **8**.[8]

Having completed the synthesis of all fragments, we then initiated their coupling (Scheme 6). The coupling began with esterification between phenylserine derivative **9** and alcohol **8** using EDCI·HCl.[15] Removal of the Fmoc protecting group followed by condensation with dipeptide **4** afforded depsipeptide **26**. Selective removal of the primary TBS protecting group and Dess−Martin oxidation followed by Pinnick oxidation gave the cyclization precursor **27**. Subsequent macrolactamization with EDCI·HCl, followed by removal of the TBS protecting group, provided Kazmaier's intermediate (**3**). After oxidation as reported by Kazmaier et al.,[8] we sought to construct the enol ether moiety of miuraenamides A (**1**) and D (**2**). Attempts to employ trimethyl orthoformate with solid acids such as

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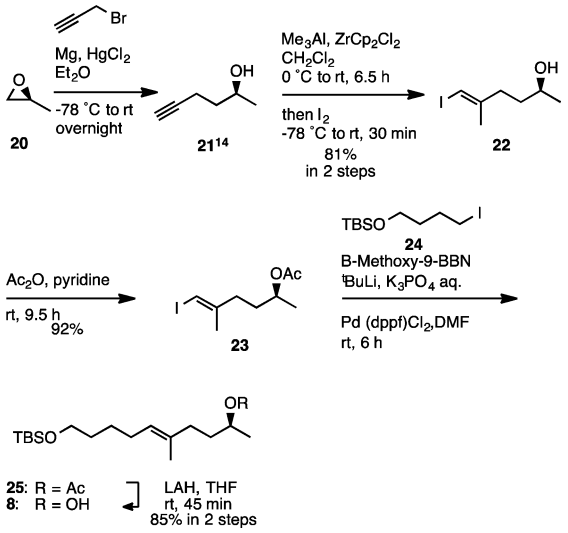
Scheme 5. Synthesis of Alcohol 8



Montmorillonite, Amberlyst, and Dowex, failed. Finally, diazomethane was found to be effective for the formation of methyl enol ether to give protected miuraenamides A (**1**) and D (**2**). Subsequent *O*-deallylation using $[CpRu(MeCN)_3]PF_6$ according to the reported procedure[8] followed by HPLC purification provided miuraenamide A (**1**) and miuraenamide D (**2**). The spectroscopic data (^1H and ^{13}C NMR, HRMS, and optical rotation) for synthetic miuraenamides A (**1**) and D (**2**) were fully consistent with those of the natural product.

The biological activities of synthetic miuraenamides A and D toward the HeLa cell line, HeLa-S3 cell line, and the anti-*Phytophthora* activity were then investigated. The biological activities of synthesized miuraenamides A and D corresponded to that of natural products (Table 1 and Figure 2).[16]

CONCLUSIONS

In conclusion, we have achieved the total synthesis of miuraenamides A (**1**) and D (**2**) via the longest linear sequence of 20 steps, while the previously reported procedure by Kazmaier required 15 steps.[8] The overall yield for miuraenamide A over the longest linear sequence is 3.2%, while the overall yield of the previously reported procedure for miuraenamide A is 1.9%.[8] We expect that this synthesis will be useful for the synthesis of miuraenamide analogues, which could contribute to the investigation of structure−activity relationships.

Table 1. Biological Activities of Synthesized 1 and 2 and Natural Products

compds	IC_{50} values toward HeLa cells (μM)	IC_{50} values toward HeLa-S3 cell line (μM)	minimum doses for anti-*Phytophthora* activity (ng/disk)[16]
1	0.031	0.38	3
2	0.021	1.32	30
nat. **1**	not reported	0.27	3
nat. **2**	not reported	not reported	30

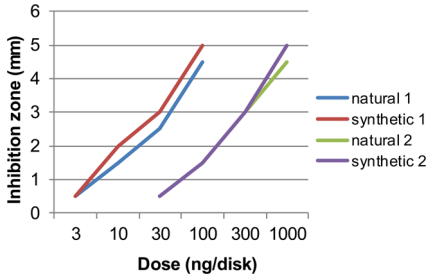
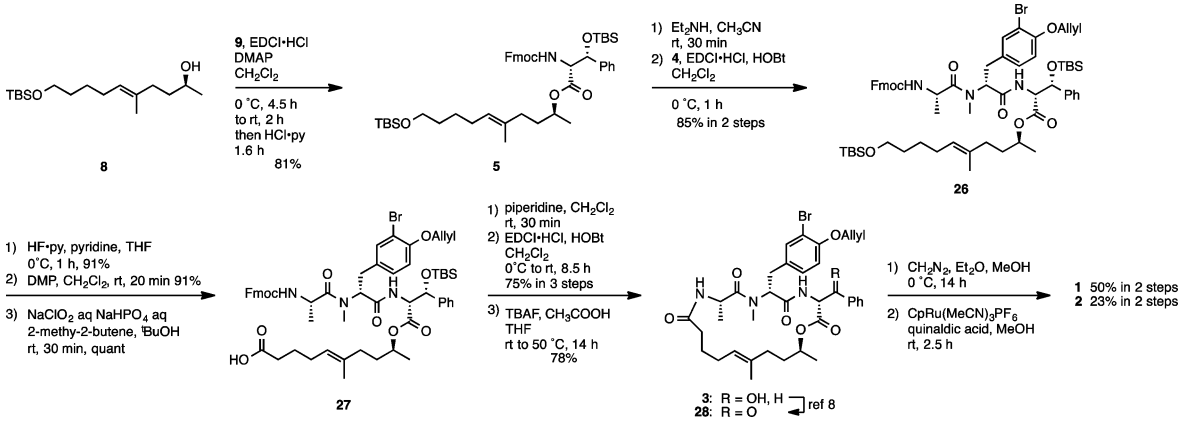


Figure 2. Anti-*Phytophthora* activity of synthesized **1** and **2** and natural products based on a paper disk assay.

EXPERIMENTAL SECTION

General Information. Chemicals and solvents were of the best grade available and were used as received from commercial sources. Optical rotations were measured with a digital polarimeter. ^1H NMR spectra were recorded on a 400 MHz NMR spectrometer. Chemical shifts are reported as δ values in parts per million relative to the residual solvent signal (CHD_2OD, δ = 3.31 ppm; $CHCl_3$, δ = 7.26 ppm; C_6HD_5, δ = 7.16 ppm for ^1H), and coupling constants are in hertz (Hz). The following abbreviations are used for spin multiplicity: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, and br = broad. ^{13}C NMR spectra were recorded on a 100 MHz NMR spectrometer using $CDCl_3$ as a solvent. Chemical shifts are reported in parts per million from the solvent signal ($CDCl_3$: δ = 77.2 ppm). IR spectra were recorded on an FT-IR spectrometer. High-resolution mass spectra (HRMS) were recorded by electrospray ionization (ESI) using time-of-flight (TOF). Reactions were monitored by thin-layer chromatography (TLC), and TLC plates were visualized either by UV detection or phosphomolybdic acid solution. Silica Gel 60N (Irregular, 63-212 μm) were used for column chromatography unless otherwise noted. Organic solvents for moisture-sensitive reactions were distilled from the following drying agents: THF (Na-benzophenone ketyl),

Scheme 6. Synthesis of Miuraenamides A (1) and D (2)



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diethyl ether (Na-benzophenone ketyl), benzene (Na), toluene (Na), and CH_2Cl_2 (P_2O_5). Anhydrous DMF was used as obtained from commercial supplies. All moisture-sensitive reactions were performed under an atmosphere of nitrogen, and the starting materials were azeotropically dried with benzene before use.

Experimental Procedure. *(9H-Fluoren-9-yl)methyl (S)-4-(methoxy(methyl)carbamoyl)-2,2-dimethyloxazolidine-3-carboxylate (**12**).* To a stirred solution of *N*-Fmoc-L-serine (2.09 g, 6.39 mmol) and *N*,*O*-dimethylhydroxylamine hydrochloride (629 mg, 6.44 mmol) in DMF (9.0 mL) at room temperature were added HATU (3.62 g, 9.52 mmol) and $^{i}Pr_2EtN$ (1.7 mL, 9.8 mmol). After stirring for 30 min, the reaction mixture was diluted with 10% aqueous citric acid and extracted with EtOAc (3 × 30 mL). The combined extracts were washed with saturated aqueous $NaHCO_3$ (50 mL) and brine (50 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by column chromatography on silica gel (70 g, chloroform−EtOAc 2:1) to give Weinreb amide **11**[9] (2.66 g, quant) as a colorless solid. Weinreb amide **11**[9] (2.66 g, 6.39 mmol) was dissolved in a mixture of acetone (20 mL) and 2, 2-dimethoxypropane (20 mL). $BF_3{\cdot}OEt_2$ (0.08 mL, 0.6 mmol) was slowly added at room temperature. After stirring for 12 h, the reaction mixture was quenched with Et_3N (3 mL), and the resulting mixture was concentrated. The residue was purified by column chromatography on silica gel (70 g, hexane−EtOAc 1:1) to give acetonide **12** (2.3 g, 88% in 2 steps) as a colorless solid: $[\alpha]_D^{23}$ −18.6 (*c* 1.01, $CHCl_3$); IR (neat, cm^{-1}) 1700, 1653, 1436, 1219, 775; ^1H NMR (400 MHz, $CDCl_3$) (rotamer ratio 3:2) major rotamer δ 7.76−7.72 (m, 2H), 7.61−7.56 (m, 2H), 7.41−7.34 (m, 2H), 7.33−7.29 (m, 2H), 4.54 (d, *J* = 5.8 Hz, 2H), 4.51 (dd, *J* = 7.2, 2.5 Hz, 1H), 4.17 (t, *J* = 5.8 Hz, 1H), 4.13 (dd, *J* = 9.2, 7.2 Hz, 1H), 3.94 (dd, *J* = 9.2, 2.5 Hz, 1H), 3.41 (s, 3H), 3.10 (s, 3H), 1.72 (s, 3H), 1.53 (s, 3H); minor rotamer (selected) δ 4.73 (dd, *J* = 7.2, 3.1 Hz, 1H), 4.72 (dd, *J* = 10.7, 4.5 Hz, 1H), 4.66 (dd, *J* = 10.7, 4.5 Hz, 1H) 4.26 (t, *J* = 4.5 Hz, 1H), 4.08 (dd, *J* = 9.2, 7.2 Hz, 1H), 3.89 (dd, *J* = 9.2, 3.1 Hz, 1H), 3.72 (s, 3H), 3.19 (s, 3H), 1.15 (s, 3H), 0.94 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) major rotamer δ 170.4, 152.1, 144.3, 143.7, 141.4, 141.3, 127.7 (2C), 127.1, 127.0, 125.0 (2C), 119.9, 119.8, 95.5, 66.5, 66.1, 61.0, 57.6, 47.5, 32.5, 24.7, 24.3; minor rotamer δ 170.2, 152.9, 144.1, 144.0, 141.7, 141.4, 127.7 (2C), 127.6, 127.3, 124.6, 124.4, 120.1, 120.0, 94.8, 66.8, 66.0, 61.2, 57.9, 47.2, 32.4, 24.9, 24.6; HRMS (ESI) *m*/*z* 411.1901, calcd for $C_{23}H_{27}N_2O_5$ $[M + H]^+$ 411.1919.

*(9H-Fluoren-9-yl)methyl (S)-4-benzoyl-2,2-dimethyloxazolidine-3-carboxylate (**13**).* To a solution of acetonide **12** (875.5 mg, 2.13 mmol) in THF (20 mL) cooled at −78 °C (dry ice/acetone bath) was added a 2 M solution of phenylmagnesium bromide in THF (4.2 mL, 8 mmol). The reaction mixture was stirred at −78 °C for 10 min, warmed up to room temperature, and stirred for 1.5 h. The mixture was cooled at 0 °C and diluted with saturated aqueous NH_4Cl (40 mL), The mixture was extracted with EtOAc (3 × 30 mL), and the combined extracts were washed with saturated aqueous $NaHCO_3$ (50 mL) and brine (50 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by column chromatography on silica gel (50 g, hexane−EtOAc 20:1, chloroform−EtOAc 20:1) to give ketone **13** (616 mg, 68%) as a colorless solid: $[\alpha]_D^{23}$ −33.7 (*c* 1.00, $CHCl_3$); IR (neat, cm^{-1}) 1653, 1635, 1436, 1340, 1219, 1095, 722; ^1H NMR (400 MHz, $CDCl_3$) (rotamer ratio 2:1) major rotamer δ 7.73 (d, *J* = 7.2 Hz, 2H), 7.67−7.55 (m, 2H), 7.51−7.30 (m, 8H), 7.06 (t, *J* = 7.3 Hz, 1H), 5.10 (dd, *J* = 9.0, 2.2 Hz, 1H), 4.49 (d, *J* = 5.6 Hz, 2H), 4.20 (t, *J* = 9.0 Hz, 1H), 4.07 (t, *J* = 5.6 Hz, 1H), 3.92 (dd, *J* = 9.0, 2.2 Hz, 1H), 1.77 (s, 3H), 1.54 (s, 3H); minor rotamer δ 7.88 (d, *J* = 7.4 Hz, 2H), 7.78 (d, *J* = 7.4 Hz, 2H), 7.18 (t, *J* = 8.1 Hz, 2H), 7.14 (t, *J* = 8.3 Hz, 2H), 5.37 (dd, *J* = 7.4, 2.9 Hz, 1H), 4.75 (dd, *J* = 10.8, 4.5 Hz, 1H), 4.68 (dd, *J* = 10.8, 4.5 Hz, 1H), 4.27 (t, *J* = 4.5 Hz, 1H), 4.22 (t, *J* = 7.4 Hz, 1H), 3.89 (dd, *J* = 7.4, 2.9 Hz, 1H), 1.20 (s, 3H), 0.98 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) major rotamer δ 194.2, 151.9, 143.95, 143.7, 141.4, 141.0, 134.7, 133.7, 128.9, 128.4 (2C), 127.6, 127.4, 127.2, 126.9 (2C), 124.9, 124.7, 119.8, 119.7, 95.6, 66.3, 66.1, 61.5, 47.3, 24.9, 24.1; minor rotamer δ 194.8, 152.8, 144.0, 143.9, 141.7, 141.5, 134.1, 133.6, 128.9, 128.4 (2C) 127.7, 127.6, 127.3, 126.9 (2C), 124.9, 124.7, 120.0 (2C), 94.9, 66.7, 65.8, 62.0, 47.2, 25.1, 24.4; HRMS (ESI) *m*/*z* 450.1681, calcd for $C_{27}H_{25}NO_4Na$ $[M + Na]^+$ 450.1681.

*(9H-Fluoren-9-yl)methyl (S)-4-((R)-hydroxy(phenyl)methyl)-2,2-dimethyloxazolidine-3-carboxylate (**14**).* To a stirred solution of ketone **13** (469 mg, 1.09 mmol) in THF (30 mL) cooled at 0 °C was added dropwise a 1.0 M solution of DIBAL in THF (2.5 mL, 2.5 mmol). After stirring at 0 °C for 1 h, the mixture was diluted with saturated aqueous Na/K tartrate (60 mL) and stirred at room temperature for 11 h. The reaction mixture was extracted with EtOAc (3 × 50 mL), and the combined extracts were washed with saturated aqueous $NaHCO_3$ (50 mL) and brine (50 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by column chromatography on silica gel (60 g, hexane−EtOAc 5:1, 3:1) to give alcohol **14** (365 mg, 78%) as a colorless solid: $[\alpha]_D^{23}$ + 1.37 (*c* 1.00, $CHCl_3$); IR (neat, cm^{-1}) 3456, 2953, 2925, 2854, 1695, 1451, 1377, 1348, 1256, 1198, 1091, 704; ^1H NMR (400 MHz, $CDCl_3$) (rotamer ratio 3:2) major rotamer δ 7.78−7.68 (m, 2H), 7.63−7.53 (m, 2H), 7.40−7.21 (m, 8H), 6.87 (m, 1H), 4.86 (m, 1H), 4.66 (m, 1H), 4.48 (m, 1H), 4.18 (m, 1H), 3.89 (m, 1H), 3.53 (m, 1H), 3.51 (m, 1H), 2.02 (m, 1H, OH), 1.62 (s, 3H), 1.35 (s, 3H); minor rotamer δ 5.05 (m, 1H), 4.26 (m, 1H), 4.14 (m, 1H), 3.69 (m, 1H), 3.31 (m, 1H, OH), 0.80 (s, 3H), 0.69 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) major rotamer δ 152.5, 144.0 (2C), 141.6, 140.7 (2C), 128.3 (2C), 127.8 (2C), 127.3 (2C), 127.2 (2C), 125.7(2C), 124.7, 120.2 (2C), 95.0, 77.6, 65.1, 63.2, 62.3, 47.9, 26.0, 23.4; minor rotamer δ 154.2, 143.7 (2C), 141.6, 140.7 (2C), 128.3 (2C), 127.8 (2C), 127.3 (2C), 127.2 (2C) 126.1 (2C), 124.7, 120.1 (2C), 94.7, 73.6, 66.4, 63.6, 63.5, 47.4, 25.2, 22.7; HRMS (ESI) *m*/*z* 430.2004, calcd for $C_{27}H_{28}NO_4$ $[M + H]^+$ 430.2018.

*(9H-Fluoren-9-yl)methyl ((1R,2S)-1,3-dihydroxy-1-phenylpropan-2-yl)carbamate (**15**).* To a stirred solution of alcohol **14** (1.08 g, 2.52 mmol) in EtOH (50 mL) warmed at 50 °C was added *p*-TsOH·H_2O (54.7 mg, 0.287 mmol). After stirring at 50 °C for 2 h, to the reaction mixture was added *p*-TsOH·H_2O (114 mg, 0.599 mmol) and stirred at 50 °C for an additional 13 h. The mixture cooled to room temperature was diluted with saturated aqueous $NaHCO_3$ (100 mL) and extracted with EtOAc (3 × 40 mL). The combined extracts were washed with brine (50 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by column chromatography on silica gel (40 g, hexane−EtOAc 2:3, 1:2) to give diol **15** (703 mg, 72%) as a colorless solid: $[\alpha]_D^{23}$ + 11.6 (*c* 0.760, $CHCl_3$); IR (neat, cm^{-1}) 3363, 2925, 1684, 1576, 1436, 1220, 1058, 755; ^1H NMR (400 MHz, $CDCl_3$) δ 7.78 (d, *J* = 7.4 Hz, 2H), 7.60 (d, *J* = 7.4 Hz, 2H), 7.43−7.30 (m, 9H), 5.62 (m, 1H, NH), 5.08 (m, 1H), 4.47−4.39 (m, 2H), 4.22 (t, *J* = 6.5 Hz, 1H), 3.87−3.82 (m, 2H), 3.65 (m, 1H), 2.92 (m, 1H, OH), 2.40 (m, 1H, OH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.6, 143.9 (2C), 141.4 (2C), 140.8, 128.7 (2C), 128.0, 127.8 (2C), 127.2 (2C), 125.8 (2C), 125.2 (2C), 120.1 (2C), 76.2, 66.9, 61.7, 56.7, 47.4; HRMS (ESI) *m*/*z* 412.1511, calcd for $C_{24}H_{23}NO_4Na$ $[M + Na]^+$ 412.1524.

*(9H-Fluoren-9-yl)methyl ((5R,6S)-2,2,3,3,9,9,10,10-octamethyl-5-phenyl-4,8-dioxa-3,9-disilaundecan-6-yl)carbamate (**16**).* To a stirred solution of diol **15** (219 mg, 0.563 mmol) in CH_2Cl_2 (6.0 mL) cooled at 0 °C were added 2,6-lutidine (0.26 mL, 2.2 mmol) and TBSOTf (0.39 mL, 1.6 mmol). After stirring at room temperature for 20 min, the reaction mixture was diluted with distilled water (20 mL) and extracted with EtOAc (3 × 40 mL). The combined extracts were washed with saturated aqueous $NaHCO_3$ (50 mL) and brine (50 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by column chromatography on silica gel (40 g, hexane−EtOAc 20:1, 10:1) to give disilyl ether **16** (355 mg, quant) as a colorless oil: $[\alpha]_D^{23}$ + 1.84 (*c* 0.976, $CHCl_3$); IR (neat, cm^{-1}) 3447, 3349, 3065, 2954, 2928, 2857, 1738, 1510, 1407, 1389, 1252, 1100, 1063, 862, 778, 758, 740, 700; ^1H NMR (400 MHz, $CDCl_3$) δ 7.75 (d, *J* = 7.4 Hz, 2H), 7.53 (d, *J* = 7.6 Hz, 1H), 7.50 (d, *J* = 7.6 Hz, 1H), 7.41−7.21 (m, 9H), 4.90 (m, 1H, NH), 4.89 (m, 1H), 4.28 (m, 1H), 4.17−4.11 (m, 2H), 3.95−3.86 (m, 2H), 3.55 (dd, *J* = 10.0, 4.1 Hz, 1H), 0.91 (s, 9H), 0.89 (s, 9H), 0.04 (s, 6H), 0.03 (s, 3H), −0.18 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.2, 144.2 (2C), 144.0, 141.4 (2C), 128.1 (2C), 127.7 (2C), 127.6 (2C), 127.1 (2C), 126.9 (2C), 125.3 (2C), 120.0, 73.8, 66.8, 61.1, 58.5, 47.2, 26.0 (3C), 25.9 (3C), 18.4, 18.3, −4.6, −5.1, −5.2, −5.4 HRMS (ESI) *m*/*z* 640.3246, calcd for $C_{36}H_{51}NO_4Si_2Na$ $[M + Na]^+$ 640.3254.

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*(9H-Fluoren-9-yl)methyl ((1R,2S)-1-((tert-Butyldimethylsilyl)oxy)-3-hydroxy-1-phenylpropan-2-yl)carbamate (**17**).* To a stirred solution of disilyl ether **16** (326 mg, 0.517 mmol) in pyridine (7.4 mL) cooled at 0 °C was added 65% HF·pyridine (1.5 mL), and the reaction mixture was stirred at 0 °C for 1 h. After stirring at room temperature for 30 min, the reaction mixture was poured into saturated aqueous $NaHCO_3$ (80 mL) at room temperature. The mixture was extracted with EtOAc (3 × 50 mL), and the combined extracts were washed with brine (50 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by column chromatography on silica gel (40 g, hexane−EtOAc 3:1) to give alcohol **17** (222 mg, 85% in 2 steps) as a colorless oil: $[\alpha]_D^{23}$ −12.5 (*c* 0.800, $CHCl_3$); IR (neat, cm^{-1}) 3441, 3327, 3065, 2954, 2928, 2857, 1710, 1471, 1405, 1338, 1252, 1058, 859, 837, 778, 758, 740, 702; ^1H NMR (400 MHz, $CDCl_3$) δ 7.78 (d, *J* = 7.3 Hz, 2H), 7.62 (t, *J* = 7.2 Hz, 2H), 7.43−7.28 (m, 9H), 5.72 (d, *J* = 7.8 Hz, 1H, NH), 5.19 (br d, *J* = 1.7 Hz, 1H), 4.47−4.38 (m, 2H), 4.24 (t, *J* = 6.8 Hz, 1H), 3.88 (br d, *J* = 11.2 Hz, 1H), 3.69 (m, 1H), 3.48 (m, 1H), 2.98 (br d, *J* = 11.2 Hz, 1H, OH), 0.93 (s, 9H), 0.04 (s, 3H), −0.11 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.2, 144.0, 143.9, 141.4 (2C), 140.9, 128.5 (2C), 127.8 (2C), 127.1 (2C), 125.9 (2C), 125.2, 125.1 (2C), 120.1 (2C), 77.7, 67.0, 61.2, 57.0, 47.3, 25.9 (3C), 18.1, −4.7, −5.2; HRMS (ESI) *m*/*z* 526.2354, calcd for $C_{30}H_{37}NO_4SiNa$ $[M + Na]^+$ 526.2389.

*(2R,3R)-2-((((9H-Fluoren-9-yl)methoxy)carbonyl)amino)-3-((tert-butyldimethylsilyl)oxy)-3-phenylpropanoic Acid (**9**).* To a stirred solution of alcohol **17** (210 mg, 0.417 mmol) in CH_3CN (4.2 mL) at room temperature were added pH 7 phosphate buffered solution (2.8 mL), TEMPO (9.2 mg, 58 μmol), 80% $NaClO_2$ (96.1 mg, 0.850 mmol), and 12% aqueous NaClO (0.4 mL). After stirring at room temperature for 1 h, the reaction mixture was diluted with saturated aqueous Na_2SO_3 (10 mL). After stirring for 10 min, the resulting mixture was acidified with 1 M aqueous HCl (30 mL) and extracted with EtOAc (3 × 20 mL). The combined extracts were washed with brine (30 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by column chromatography on silica gel (45 g, chloroform− methanol 50:1, 10:1) to give phenyl serine derivative **9** (207 mg, 96%) as a colorless oil: $[\alpha]_D^{23}$ −47.0 (*c* 0.996, $CHCl_3$); IR (neat, cm^{-1}) 3437, 3066, 2954, 2929, 2857, 1725, 1520, 1472, 1463, 1422, 1361, 1340, 1253, 1216, 1104, 1071, 839, 778, 757, 700; ^1H NMR (400 MHz, $CDCl_3$) δ 7.77 (d, *J* = 7.3 Hz, 2H), 7.57 (t, *J* = 6.8 Hz, 2H), 7.42−7.29 (m, 9H), 5.40 (d, *J* = 8.3 Hz, 1H, NH), 5.17 (d, *J* = 3.4 Hz, 1H), 4.70 (dd, *J* = 8.3, 3.4 Hz, 1H), 4.44−4.31 (m, 2H), 4.22 (t, *J* = 6.8 Hz, 1H), 0.92 (s, 9H), 0.09 (s, 3H), −0.09 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.6, 155.7, 143.8 (2C), 141.4 (2C), 139.7, 128.3 (2C), 128.2 (2C), 127.8 (2C), 127.1 (2C), 126.4 (2C), 125.2, 120.1 (2C), 75.3, 67.3, 61.2, 47.1, 25.8 (3C), 18.2, −4.7, −5.1; HRMS (ESI) *m*/*z* 518.2385, calcd for $C_{30}H_{36}NO_5Si$ $[M + H]^+$ 518.2362.

*Methyl (R)-2-((S)-2-((((9H-Fluoren-9-yl)methoxy)carbonyl)-amino)-N-methylpropanamido)-3-(4-(allyloxy)-3-bromophenyl)-propanoate (**19**).* To a stirred solution of bromotyrosine derivative 7[8] (547 mg, 1.28 mmol) in CH_2Cl_2 (2.6 mL) at room temperature was added trifluoroacetic acid (1.3 mL, 16.9 mmol). After stirring for 1 h, the reaction mixture was concentrated to give crude amine·TFA. To a stirred solution of the crude amine·TFA in DMF (3.8 mL) at room temperature were added *N*-Fmoc-L-alanine (**6**) (597 mg, 1.92 mmol), DIPEA (1.5 mL, 8.7 mmol), and HATU (727 mg, 1.91 mmol). After stirring for 1 h, the reaction mixture was diluted with 10% aqueous citric acid (20 mL) and extracted with EtOAc (30 mL). The organic layer was washed with saturated aqueous $NaHCO_3$ (20 mL) and brine (20 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by column chromatography on silica gel (44 g, hexane−EtOAc 3:2) to give dipeptide **19** (782 mg, 98%) as a colorless solid: $[\alpha]_D^{23}$ + 27.8 (*c* 1.01, $CHCl_3$); IR (neat, cm^{-1}) 3414, 3316, 3066, 3017, 2952, 1734, 1652, 1604, 1499, 1451, 1411, 1349, 1250, 1050, 1017, 997, 804, 742; ^1H NMR (400 MHz, $CDCl_3$) δ 7.76 (d, *J* = 7.3 Hz, 2H), 7.59 (d, *J* = 7.3 Hz, 2H), 7.40 (t, *J* = 7.3 Hz, 2H), 7.35 (d, *J* = 2.0 Hz, 1H), 7.31 (t, *J* = 7.3 Hz, 2H), 7.07 (dd, *J* = 8.7, 2.0 Hz, 1H), 6.80 (d, *J* = 8.7 Hz, 1H), 6.03 (ddt, *J* = 17.1, 10.7, 4.9 Hz, 1H), 5.76 (d, *J* = 7.3 Hz, 1H, NH), 5.44 (d, *J* = 17.1 Hz, 1H), 5.33−5.27 (m, 2H), 4.57 (m, 1H), 4.56 (d, *J* = 4.9 Hz, 2H), 4.34 (d, *J* = 7.3 Hz, 2H), 4.20 (t, *J* = 7.3 Hz, 1H), 3.76 (s, 3H), 3.34 (dd, *J* = 4.9, 14.9 Hz, 1H), 2.95 (dd, *J* = 14.9, 11.7 Hz, 1H), 2.89 (s, 3H), 1.00 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.4, 170.7, 155.5, 153.9, 144.0, 143.9, 141.3 (2C), 133.6, 132.5, 130.3, 128.7, 127.8 (2C), 127.1 (2C), 125.3 (2C), 120.0 (2C), 117.9, 113.7, 112.2, 77.3, 69.8, 67.0, 58.0, 52.6, 47.2, 33.6, 32.5, 18.7; HRMS (ESI) *m*/*z* 621.1633, calcd for $C_{32}H_{34}{}^{79}BrN_2O_6$ $[M + H]^+$ 621.1600.

*(R)-2-((S)-2-((((9H-Fluoren-9-yl)methoxy)carbonyl)amino)-N-methylpropanamido)-3-(4-(allyloxy)-3-bromophenyl)propanoic Acid (**4**).* To a stirred solution of dipeptide **19** (156 mg, 0.251 mmol) in THF (6.3 mL) cooled at 0 °C was added dropwise a solution of $LiOH·H_2O$ (32.6 mg, 0.776 mol) in water (5.0 mL) cooled at 0 °C for 10 min and stirred at 0 °C for 20 min. The reaction mixture was acidified with 1 M aqueous HCl (20 mL) and extracted with EtOAc (3 × 10 mL). The combined extracts were washed with brine (15 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by column chromatography on silica gel (18 g, chloroform−EtOAc 2:1; chloroform−methanol 15:1, 10:1) to give dipeptide **4** (130 mg, 86%) as a colorless solid: $[\alpha]_D^{23}$ + 15.5 (*c* 1.00, $CHCl_3$); IR (neat, cm^{-1}) 3402, 3323, 2931, 1714, 1646, 1610, 1496, 1451, 1411, 1239, 1047, 932, 756, 740; ^1H NMR (400 MHz, $CDCl_3$) (rotamer ratio 9:1) major rotamer δ 7.75 (d, *J* = 7.8 Hz, 2H), 7.59 (d, *J* = 7.2 Hz, 2H), 7.41−7.28 (m, 5H), 7.06 (br d, 10.2 Hz, 1H), 6.79 (d, 8.8 Hz, 1H), 6.03 (ddt, *J* = 17.1, 10.2, 4.9 Hz, 1H), 5.76 (d, *J* = 7.3 Hz, 1H, NH), 5.44 (d, *J* = 17.1 Hz, 1H), 5.29 (d, *J* = 10.2 Hz, 1H), 5.21 (dd, *J* = 11.5, 4.9 Hz, 1H), 4.58 (m, 1H), 4.56 (d, *J* = 4.9 Hz, 2H), 4.35 (d, *J* = 7.3 Hz, 2H), 4.21 (t, *J* = 7.3 Hz, 1H), 3.35 (dd, *J* = 14.4, 4.9 Hz, 1H), 3.02 (dd, *J* = 14.4, 11.5 Hz, 1H), 2.90 (s, 3H), 1.05 (d, *J* = 6.3 Hz, 3H) minor rotamer (selected) δ 2.95−2.87 (m, 1H), 2.81 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.5, 174.1, 156.1, 153.7, 144.0, 143.8, 141.3 (2C), 133.4, 132.6, 131.0, 128.8, 127.8 (2C), 127.2 (2C), 125.3 (2C), 120.0 (2C), 117.8, 113.6, 112.1, 77.3, 69.7, 67.2, 47.3, 47.1, 33.5, 33.0, 17.9 ; HRMS (ESI) *m*/*z* 607.1442, calcd for $C_{31}H_{32}{}^{79}BrN_2O_6$ $[M + H]^+$ 607.1443.

*(S)-Hex-5-yn-2-ol (**21**).* To a stirred suspension of magnesium turnings (7.1 g, 290 mmol) in dry ether (140 mL) with mercury(II) chloride (612 mg, 2.25 mmol) and a crystal of I_2 (28.0 mg, 0.110 mmol) cooled at 0 °C was slowly added a 80 wt % solution of propargyl bromide in toluene (15.0 mL, 132 mmol). The mixture was stirred at 0 °C for 1 h and then warmed to room temperature and stirred for 1 h. To a solution of epoxide (**20**) (2.49 g, 42.8 mmol) in ether (300 mL) cooled at −78 °C was slowly added a 0.94 M solution of propargyl magnesium bromide in ether (132 mmol, prepared as above). After stirring at −78 °C for 30 min, the reaction mixture was slowly warmed to room temprature, stirred overnight, quenched at 0 °C with saturated aqueous NH_4Cl solution (300 mL), and extracted with Et_2O (5 × 100 mL). The combined extracts were washed with brine (200 mL), dried (Na_2SO_4), and distilled under reduced pressure (bp 38−63 °C, 1.7 kPa) to give alkyne **21** (4.80 g, crude). Spectral data are consistent with that reported.[3]

*(S,E)-6-Iodo-5-methylhex-5-en-2-ol (**22**).* To a suspension of $ZrCp_2Cl_2$ (7.72 g, 26.6 mmol) in CH_2Cl_2 (13 mL) cooled at 0 °C was added a 2.0 M solution of trimethylalminium in toluene (24.5 mL, 49 mmol), and the reaction mixture was stirred at room temperature for 2.5 h. To the stirred solution cooled at −20 °C was added alkyne **21** (2.06 g, crude), and the reaction mixture was stirred at room temperature for 6.5 h. After cooling to −78 °C, a solution of iodine (5.70 g, 22.4 mmol) in THF (10 mL) was added, and the mixture was warmed to room temperature and stirred for 30 min. The mixture was diluted with saturated aqueous Na/K tartrate (80 mL), stirred overnight, and extracted with Et_2O (5 × 100 mL). The combined extracts were washed with brine (50 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by column chromatography on silica gel (130 g, hexane−EtOAc 8:1, 6:1) to give vinyl iodide **22** (3.36 g, 81%) as a colorless oil: $[\alpha]_D^{23}$ + 11.7 (*c* 0.950, $CHCl_3$); IR (neat, cm^{-1}) 3348, 2964, 2926, 2854, 1616, 1457, 1375, 1268, 1129, 932, 771, 666; ^1H NMR (400 MHz, $CDCl_3$) δ 5.93 (d, *J* = 1.1 Hz, 1H), 3.78 (sext, *J* = 6.1 Hz, 1H), 2.38−2.23 (m, 2H), 1.85 (d, *J* = 1.1 Hz, 3H), 1.61−1.55 (m, 2H), 1.20 (d, *J* = 6.1 Hz, 3H); ^{13}C NMR (100

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MHz, $CDCl_3$) δ 147.8, 75.0, 67.5, 37.1, 35.9, 24.0, 23.8; HRMS (ESI) m/z 241.0080, calcd for $C_7H_{14}IO$ $[M + H]^+$ 241.0089.

*(S,E)-6-Iodo-5-methylhex-5-en-2-yl Acetate (**23**).* To a stirred solution of vinyl iodide **22** (5.85 g, 24.4 mmol) in pyridine (24 mL) at room temperature was added Ac_2O (7.0 mL, 74 mmol). After stirring for 9.5 h, the reaction mixture was diluted with distilled water (60 mL) and extracted with EtOAc (3 × 50 mL). The combined extracts were washed with 1 M hydrochloric acid (50 mL) and brine (50 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by column chromatography on silica gel (86 g, hexane−EtOAc 100:1, 20:1) to give acetate **23** (6.33g, 92%) as a colorless oil: $[\alpha]_D^{23}$ −9.18 (*c* 1.00, $CHCl_3$); IR (neat, cm^{-1}) 2976, 2938, 1734, 1644, 1445, 1372, 1241, 1142, 1077, 1018, 949, 775; ^1H NMR (400 MHz, $CDCl_3$) δ 5.88 (d, *J* = 1.0 Hz, 1H), 4.84 (sext, *J* = 6.4 Hz, 1H), 2.27−2.15 (m, 2H), 2.01 (s, 3H), 1.82 (br s, 3H), 1.73 (m, 1H), 1.61 (m, 1H), 1.20 (d, *J* = 6.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.7, 147.1, 75.1, 70.3, 35.6, 33.9, 23.9, 21.4, 20.0; HRMS (ESI) m/z 305.0033, calcd for $C_9H_{15}IO_2Na$ $[M + Na]^+$ 305.0014.

*(S,E)-10-((tert-Butyldimethylsilyl)oxy)-5-methyldec-5-en-2-yl Acetate (**25**).* To alkyl iodide **24** (986 mg, 3.13 mmol) was added a 1.0 M solution of B-MeO-9-BBN in hexane (4.2 mL, 4.2 mmol), and the mixture was cooled at −78 °C. To this solution was added a 1.38 M solution of *tert*-butyllithium in pentane (4.0 mL, 5.5 mmol), and the mixture at −78 °C was stirred for 5 min, then warmed to room temperature and stirred for 1.5 h. To this solution were added saturated aqueous K_3PO_4 (0.4 mL, 1.6 mmol) and a solution of acetate **23** (295 mg, 1.04 mmol) in DMF (4.0 mL), and the mixture was stirred under a steam of N_2 in the dark for 2.5 h. To the reaction mixture was added Pd(dppf)Cl_2 (76.8 mg, 0.104 mmol), and the mixture was stirred for 5 h. An additional amount of Pd(dppf)Cl_2 (20.7 mg, 0.0282 mmol) was added to the reaction mixture. After stirring for 1 h, the reaction mixture was diluted with brine (40 mL) and extracted with EtOAc (3 × 40 mL). The combined extracts were washed with saturated aqueous $NaHCO_3$ (50 mL) and brine (50 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by column chromatography on silica gel (57 g, hexane−EtOAc 50:1, 20:1) to give coupling product **25** (623 mg, crude) as a colorless oil:

*(S,E)-10-((tert-Butyldimethylsilyl)oxy)-5-methyldec-5-en-2-ol (**8**).* To a stirred solution of coupling product **25** (623 mg, crude) in THF (5.0 mL) at room temperature was added a 1.0 M solution of LAH in THF (1.4 mL, 1.4 mmol). After stirring for 45 min, the reaction mixture was diluted with Na/K tartrate (70 mL), stirred for 3 h, and extracted with EtOAc (40 mL, 2 × 20 mL). The combined extracts were washed with saturated aqueous $NaHCO_3$ (50 mL) and brine (50 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by column chromatography on silica gel (27 g, hexane−EtOAc 20:1, 4:1) to give fatty acid moiety **8** (267 mg, 85% in 2 steps) as a colorless oil. Spectral data are consistent with that of the compound reported in the literature.[2]

*(S,E)-10-((tert-Butyldimethylsilyl)oxy)-5-methyldec-5-en-2-yl (2R,3R)-2-((((9H-Fluoren-9-yl)methoxy)carbonyl)amino)-3-((tert-butyldimethylsilyl)oxy)-3-phenylpropanoate (**5**).* To a stirred solution of *S* alcohol **8** (79.2 mg, 0.263 mmol) and phenyl serine derivative **9** (50.1 mg, 0.0967 mmol) in CH_2Cl_2 (0.4 mL) cooled at 0 °C were added successively EDCI·HCl (35.0 mg, 0.182 mmol) and DMAP (5.2 mg, 0.0425 mmol). After stirring for 4.5 h, the reaction mixture was warmed to room temperature and stirred for 2 h. To the reaction mixture was added HCl·py (6.9 mg, 0.0597 mmol). After stirring for 30 min, to the mixture cooled at 0 °C were added EDCI·HCl (11.2 mg, 0.0584 mmol) and CH_2Cl_2 (0.2 mL), then the mixture was warmed to room temperature. After stirring for 40 min, to the mixture was added HCl·py (10.0 mg, 0.0865 mmol). After stirring for 1 h, and the reaction mixture was diluted with 10% aqueous citric acid (10 mL) and extracted with EtOAc (20 mL). The organic layer was washed with saturated aqueous $NaHCO_3$ (10 mL) and brine (10 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by column chromatography on silica gel (11 g, hexane−EtOAc 25:1, 3:1) to give ester **5** (62.9 mg, 81%) as a colorless oil: $[\alpha]_D^{23}$ −24.4 (*c* 1.00, $CHCl_3$); IR (neat, cm^{-1}) 3437, 3343, 3066, 2929, 2857, 1730, 1612, 1528, 1472, 1451, 1388, 1361, 1340, 1255, 1206, 1104, 1071, 1006, 938, 837, 777, 758, 740, 700; ^1H NMR (400 MHz, $CDCl_3$) δ 7.77 (d, *J* = 7.3 Hz, 2H), 7.61 (t, *J* = 6.6 Hz, 2H), 7.42−7.23 (m, 9H), 5.63 (d, *J* = 7.8 Hz, 1H, NH), 5.18 (d, *J* = 2.9 Hz, 1H), 5.00 (t, *J* = 6.8 Hz, 1H), 4.78 (sext, *J* = 6.4 Hz, 1H), 4.60 (dd, *J* = 7.8, 2.9 Hz, 1H), 4.40 (dd, *J* = 8.3, 7.3 Hz, 1H), 4.36 (dd, *J* = 8.3, 7.3 Hz, 1H), 4.24 (t, *J* = 7.3 Hz, 1H), 3.58 (t, *J* = 6.4 Hz, 2H), 1.93 (q, *J* = 6.8 Hz, 2H), 1.78−1.70 (m, 2H), 1.52−1.38 (m, 4H), 1.48 (s, 3H), 1.32 (quint, *J* = 7.3 Hz, 2H), 1.14 (d, *J* = 6.4 Hz, 3H), 0.92 (s, 9H), 0.88 (s, 9H), 0.06 (s, 3H), 0.04 (s, 6H), −0.09 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.9, 155.7, 144.0, 143.9, 141.4 (2C), 140.5, 134.1, 128.0 (2C), 127.8 (2C), 127.6, 127.1 (2C), 126.3 (2C), 125.3 (2C), 125.0, 120.1 (2C), 75.5, 72.6, 67.2, 63.3, 61.4, 47.2, 35.2, 34.1, 32.6, 27.7, 26.1 (3C), 25.8 (4C), 19.8, 18.3 (2C), 15.9, −4.6 (2C), −5.0, −5.1; HRMS (ESI) m/z 822.4575, calcd for $C_{47}H_{69}NO_6Si_2Na$ $[M + Na]^+$ 822.4561.

*(S,E)-10-((tert-Butyldimethylsilyl)oxy)-5-methyldec-5-en-2-yl (5S,8R,11R)-8-(4-(Allyloxy)-3-bromobenzyl)-11-((R)-((tert-butyldimethylsilyl)oxy) (Phenyl)methyl)-1-(9H-fluoren-9-yl)-5,7-dimethyl-3,6,9-trioxo-2-oxa-4,7,10-triazadodecan-12-oate (**26**).* To a stirred solution of ester **5** (147 mg, 0.183 mmol) in MeCN (3.6 mL) at room temperature was added Et_2NH (1.8 mL, 17.2 mmol). After stirring for 30 min, the reaction mixture was concentrated to give crude amine. To a solution of the crude amine and dipeptide **4** (161 mg, 0.264 mmol) in CH_2Cl_2 (0.6 mL) cooled at 0 °C were added HOBt (34.8 mg, 0.257 mmol) and EDCI·HCl (107 mg, 0.559 mmol). After stirring at 0 °C for 1 h, the reaction mixture was diluted with 10% aqueous citric acid (20 mL) and extracted with EtOAc (40 mL). The organic layer was washed with saturated aqueous $NaHCO_3$ (20 mL) and brine (20 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by column chromatography on silica gel (18 g, hexane−EtOAc 10:1, 5:1) to give depsipeptide **26** (182.4 mg, 85%) as a colorless oil: $[\alpha]_D^{23}$ + 4.76 (*c* 0.976, $CHCl_3$); IR (neat, cm^{-1}) 3430, 3305, 3067, 3023, 2954, 2929, 2857, 1730, 1680, 1653, 1599, 1496, 1451, 1256, 1186, 1101, 837, 779, 758, 740, 700; ^1H NMR (400 MHz, $CDCl_3$) δ 7.76 (d, *J* = 7.6 Hz, 2H), 7.63 (d, *J* = 7.6 Hz, 2H), 7.41 (t, *J* = 7.6 Hz, 2H), 7.32−7.18 (m, 8H), 7.00 (dd, *J* = 8.5, 2.0 Hz, 1H), 6.75 (d, *J* = 8.5 Hz, 1H), 6.44 (d, *J* = 9.0 Hz, 1H, NH), 6.01 (ddt, *J* = 17.2, 10.6, 4.9 Hz, 1H), 5.82 (d, *J* = 7.4 Hz, 1H, NH), 5.42 (dt, *J* = 17.2, 1.5 Hz, 1H), 5.35 (dd, *J* = 11.1, 5.5 Hz, 1H), 5.27 (dt, *J* = 10.6, 1.5 Hz, 1H), 5.06 (t, *J* = 6.5 Hz, 1H), 4.99 (d, *J* = 5.8 Hz, 1H), 4.87−4.80 (m, 2H), 4.54 (dt, *J* = 4.9, 1.5 Hz, 2H), 4.45 (dq, *J* = 7.4, 6.7 Hz, 1H), 4.42 (d, *J* = 7.2 Hz, 2H), 4.24 (t, *J* = 7.2 Hz, 1H), 3.59 (t, *J* = 6.5 Hz, 2H), 3.07 (dd, *J* = 15.2, 5.5 Hz, 1H), 2.89 (dd, *J* = 15.2, 11.1 Hz, 1H), 2.58 (s, 3H), 1.98 (t, *J* = 7.4 Hz, 1H), 1.95 (t, *J* = 7.4 Hz, 1H), 1.87 (dt, *J* = 6.5, 5.8 Hz, 2H), 1.69−1.59 (m, 2H), 1.54 (s, 3H), 1.52−1.45 (m, 2H), 1.38−1.30 (m, 2H), 1.24 (d, *J* = 6.3 Hz, 3H), 0.91 (d, *J* = 6.7 Hz, 3H), 0.88 (s, 9H), 0.79 (s, 9H), 0.04 (s, 6H), −0.03 (s, 3H), −0.23 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.9, 169.6, 168.6, 155.5, 153.8, 144.0 (2C), 143.9 (2C), 141.4, 140.2, 134.1, 133.4, 132.5, 130.2, 128.7, 128.2 (2C), 127.8 (2C), 127.2 (2C), 126.9 (2C), 125.2 (2C), 125.1, 120.1 (2C), 117.9, 113.7, 112.2, 77.3 75.3, 72.7, 69.8, 67.1, 63.3, 59.0, 56.2, 47.4, 35.3, 34.2, 32.6, 32.1 30.2, 27.8, 26.1 (3C), 25.8, 25.7 (3C), 19.8, 18.6, 18.5, 18.1, 16.0, −4.6, −5.1 (2C), −5.2; HRMS (ESI) m/z 1166.5323, calcd for $C_{63}H_{89}{}^{79}BrN_3O_9Si_2$ $[M + H]^+$ 1166.5320.

*(S,E)-10-Hydroxy-5-methyldec-5-en-2-yl (5S,8R,11R)-8-(4-(Allyloxy)-3-bromobenzyl)-11-((R)-((tert-butyldimethylsilyl)oxy) (Phenyl)methyl)-1-(9H-fluoren-9-yl)-5,7-dimethyl-3,6,9-trioxo-2-oxa-4,7,10-triazadodecan-12-oate (**SI1**).* To a stirred solution of depsipeptide **26** (21.7 mg, 0.0185 mmol) in pyridine (0.4 mL) cooled at 0 °C was added 65% HF·pyridine (0.1 mL). After stirring for 30 min, THF (0.1 mL) was added to the reaction mixture. After stirring at 0 °C for 30 min, the reaction mixture was poured into saturated aqueous $NaHCO_3$ (10 mL) at 0 °C and extracted with EtOAc (2 × 20 mL). The combined extracts were washed with 1 M HCl (10 mL) and brine (10 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by column chromatography on silica gel (9 g, hexane−EtOAc 3:2) to give alcohol **SI1** (17.7 mg, 91%) as a colorless oil: $[\alpha]_D^{23}$ + 12.8 (*c* 0.980, $CHCl_3$); IR (neat, cm^{-1}) 3322, 2928, 2856, 1734, 1718, 1700, 1684, 1652, 1496, 1456, 1255, 1184, 1065, 837, 777, 757, 740; ^1H NMR (400 MHz, $CDCl_3$) δ 7.76 (d, *J* = 7.6 Hz, 2H), 7.64 (dd, *J* = 7.3, 3.4 Hz, 2H), 7.39 (t, *J* = 7.6 Hz, 2H), 7.31 (dd, *J* =

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7.6, 7.3 Hz, 2H), 7.28−7.18 (m, 6H), 6.99 (dd, J = 8.5, 1.4 Hz, 1H), 6.75 (d, J = 9.0 Hz, 1H, NH), 6.73 (d, J = 8.5 Hz, 1H), 6.00 (ddt, J = 17.1, 10.7, 4.9 Hz, 1H), 5.83 (d, J = 7.3 Hz, 1H, NH), 5.41 (d, J = 17.1 Hz, 1H), 5.33 (dd, J = 10.6, 5.6 Hz, 1H), 5.27 (d, J = 10.7 Hz, 1H), 4.97 (d, J = 6.8 Hz, 1H), 4.94 (t, J = 6.8 Hz, 1H), 4.87 (dd, J = 9.0, 6.8 Hz, 1H), 4.81 (m, 1H), 4.53 (d, J = 4.9 Hz, 2H), 4.48−4.39 (m, 3H), 4.25 (dd, J = 7.3, 7.2 Hz, 1H), 3.67−3.59 (m, 2H), 3.08 (dd, J = 15.0, 5.6 Hz, 1H), 2.81 (dd, J = 15.0, 10.6 Hz, 1H), 2.46 (s, 3H), 2.01−1.95 (m, 2H), 1.91−1.87 (m, 2H), 1.64 (m, 1H), 1.58−1.48 (m, 3H), 1.52 (s, 3H), 1.40 (quint, J = 7.6 Hz, 2H), 1.25 (d, J = 6.1 Hz, 3H), 0.91 (d, J = 6.8 Hz, 3H), 0.79 (s, 9H), −0.05 (s, 3H), −0.28 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.0, 169.9, 168.3, 155.6, 153.7, 144.0 (2C), 143.8 (2C), 141.3, 140.3, 133.8, 133.5, 132.5, 130.3, 128.7 (2C), 128.1 (2C), 127.7 (2C), 127.1 (3C), 125.5, 125.2 (2C), 120.0 (2C), 117.8, 113.6, 112.1, 75.1, 71.8, 69.7, 67.1, 62.7, 58.6, 56.4, 47.3, 47.2, 35.2, 33.6, 32.3 (2C), 30.0, 27.5, 25.9, 25.7(3C), 20.1, 18.3, 18.0, 15.7, −4.7, −5.2; HRMS (ESI) m/z 1052.4453, calcd for $C_{57}H_{75}{}^{79}BrN_3O_9Si$ $[M + H]^+$ 1052.4456.

*(S,E)-5-Methyl-10-oxodec-5-en-2-yl (5S,8R,11R)-8-(4-(Allyloxy)-3-bromobenzyl)-11-((R)-((tert-butyldimethylsilyl)oxy) (Phenyl)-methyl)-1-(9H-fluoren-9-yl)-5,7-dimethyl-3,6,9-trioxo-2-oxa-4,7,10-triazadodecan-12-oate (**SI2**).* To a stirred solution of alcohol **SI1** (15.7 mmol, 14.9 μmol) in CH_2Cl_2 (0.2 mL) at room temperature was added Dess−Martin periodinane (12.9 mg, 30.4 μmol). After stirring for 20 min, the reaction mixture was diluted with saturated aqueous sodium thiosulfate (5 mL), stirred for 10 min, and extracted with EtOAc (3 × 5 mL). The combined extracts were washed with brine (15 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by PLC (hexane−EtOAc 3:2) to give aldehyde **SI2** (14.3 mg, 91%) as a colorless oil: $[\alpha]_D{}^{23}$ + 10.2 (c 0.426, $CHCl_3$); IR (neat, cm^{-1}) 3419, 3325, 3061, 2977, 2930, 2857, 2715, 1725, 1690, 1653, 1603, 1496, 1451, 1409, 1256, 1185, 1082, 838, 780, 758, 740, 700; ^{1}H NMR (400 MHz, $CDCl_3$) (rotamer ratio 2:1) major rotamer δ 9.75 (t, J = 1.8 Hz, 1H), 7.77 (d, J = 7.4 Hz, 2H), 7.64 (dd, J = 7.3, 1.6 Hz, 2H), 7.4 (t, J = 7.4 Hz, 2H), 7.32−7.18 (m, 8H), 7.01 (dd, J = 8.3, 2.0 Hz, 1H), 6.76 (d, J = 8.3 Hz, 1H), 6.47 (d, J = 9.0 Hz, 1H, NH), 6.01 (ddt, J = 17.3, 10.6, 5.2 Hz, 1H), 5.82 (d, J = 7.2 Hz, 1H, NH), 5.43 (td, J = 17.3, 1.6 Hz, 1H), 5.36 (dd, J = 11.1, 5.7 Hz, 1H), 5.28 (td, J = 10.6, 1.6 Hz, 1H), 5.03 (t, J = 6.5 Hz, 1H), 4.99 (d, J = 6.1 Hz, 1H), 4.87−4.79 (m, 2H), 4.54 (dt, J = 5.2, 1.6 Hz, 2H), 4.48−4.41 (m, 3H), 4.24 (t, J = 7.2 Hz, 1H), 3.09 (dd, J = 15.2, 5.7 Hz, 1H), 2.87 (dd, J = 15.2, 11.1 Hz, 1H), 2.56 (s, 3H), 2.40 (td, J = 7.3, 1.8 Hz, 2H), 2.00 (q, J = 7.2 Hz, 2H), 1.90−1.85 (m, 2H), 1.69−1.61 (m, 4H), 1.53 (s, 3H), 1.24 (d, J = 6.3 Hz, 3H), 0.92 (d, J = 6.7 Hz, 3H), 0.80 (s, 9H), −0.04 (s, 3H), −0.25 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 203.0, 173.9, 169.6, 168.6, 155.5, 153.8, 144.1 (2C), 143.9 (2C), 141.4, 140.2, 135.4, 133.5, 132.6, 130.3, 128.7, 128.2 (2C), 127.8 (2C), 127.2 (2C), 127.0 (2C), 125.3 (2C), 123.9, 120.1 (2C), 117.9, 113.7, 112.2, 77.3, 75.3, 72.4, 69.8, 67.1, 59.0, 56.3, 47.4, 43.4, 35.2, 34.0, 32.2, 30.2, 27.3, 25.7 (3C), 22.2, 19.9, 18.6, 18.1, 16.0, −4.6, −5.1; HRMS (ESI) m/z 1072.4109, calcd for $C_{57}H_{72}{}^{79}BrN_3O_9SiNa$ $[M + Na]^+$ 1072.4118.

*(5S,8R,11R,14S,E)-8-(4-(Allyloxy)-3-bromobenzyl)-11-((R)-((tert-butyldimethylsilyl)oxy) (phenyl)methyl)-1-(9H-fluoren-9-yl)-5,7,14,17-tetramethyl-3,6,9,12-tetraoxo-2,13-dioxa-4,7,10-triazadocos-17-en-22-oic Acid (**27**).* To a stirred solution of aldehyde **SI2** (36.4 mg, 34.6 μmol) in ^{t}BuOH (1.0 mL) at room temperature were added 2-methyl-2-butene (1.0 mL, 9.4 mmol), 1 M aqueous NaH_2PO_4 solution (0.3 mL), and 1 M aqueous $NaClO_2$ solution (0.3 mL). After stirring for 30 min, the reaction mixture was diluted with saturated aqueous $NaSO_3$ (1 mL), stirred for 10 min, acidified with 1 M HCl (5 mL), and extracted with EtOAc (3 × 10 mL). The combined extracts were washed with brine (15 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by column chromatography on silica gel (10 g, chloroform−methanol−acetic acid 300:1:1) to give cyclization precursor **27** (42.0 mg, quant) as a colorless oil: $[\alpha]_D{}^{23}$ + 11.3 (c 1.00, $CHCl_3$); IR (neat, cm^{-1}) 3314, 2930, 2857, 1730, 1696, 1652, 1521, 1496, 1451, 1410, 1256, 1100, 837, 778, 759, 741, 702; ^{1}H NMR (400 MHz, $CDCl_3$) (rotamer ratio 10:1) major rotamer δ 7.77 (d, J = 7.8 Hz, 2H), 7.64 (dd, J = 7.6, 3.9 Hz, 2H), 7.40 (t, J = 7.8 Hz, 2H), 7.33−7.20 (m, 8H), 7.00 (d, J = 6.3 Hz, 1H), 6.75 (d, J = 8.3 Hz, 1H), 6.60 (d, J = 8.8 Hz, 1H, NH), 6.03 (m, 1H), 5.99 (m, 1H, NH), 5.42 (d, J = 17.6 Hz, 1H), 5.36 (dd, J = 11.2, 5.4 Hz, 1H), 5.27 (d, J = 10.7 Hz, 1H), 5.00−4.97 (m, 2H), 4.89−4.81 (m, 2H), 4.54 (d, J = 4.9 Hz, 2H), 4.48−4.37 (m, 3H), 4.24 (t, J = 7.3 Hz, 1H), 3.12 (dd, J = 15.2, 5.4 Hz, 1H), 2.80 (dd, J = 15.2, 11.2 Hz, 1H), 2.52 (s, 3H), 2.32 (t, J = 7.3 Hz, 2H), 2.04−2.01 (m, 2H), 1.88−1.83 (m, 2H), 1.73- 1.56 (m, 4H), 1.53 (s, 3H), 1.24 (d, J = 6.8 Hz, 3H), 0.92−0.86 (m, 3H), 0.80 (s, 9H), −0.04 (s, 3H), −0.26 (s, 3H); minor rotamer (selected) δ 7.57 (dd, J = 8.0, 7.2 Hz, 2H), 6.68 (d, J = 8.3 Hz, 1H), 6.50 (d, J = 7.8 Hz, 1H), 5.90 (d, J = 7.3 Hz, 1H, NH), 5.83−5.73 (m, 1H), 5.07 (d, J = 9.3 Hz, 1H), 0.89 (s, 9H), 0.06 (s, 3H), −0.10 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.9, 174.3, 169.8, 168.5, 155.7, 153.8, 144.1 (2C), 143.8 (2C), 141.4, 140.2, 135.2, 133.5, 132.6, 130.3, 128.7, 128.2 (2C), 127.8 (2C), 127.2 (2C), 126.4 (2C), 125.3 (2C),124.0, 120.0 (2C), 117.8, 113.7, 112.2, 77.3, 75.2, 72.3, 69.8, 67.2, 58.9, 56.5, 47.2, 35.2, 33.9, 33.4, 32.3, 30.3, 27.3, 25.7 (3C), 24.7, 20.0, 18.3, 18.1, 15.9, −4.6, −5.1; HRMS (ESI) m/z 1066.4246 calcd for $C_{57}H_{73}{}^{79}BrN_3O_{10}Si$ $[M + H]^+$ 1066.4248.

*(3R,6R,9S,19S,E)-6-(4-(Allyloxy)-3-bromobenzyl)-3-((R)-((tert-butyldimethylsilyl)oxy) (Phenyl)methyl)-7,9,16,19-tetramethyl-1-oxa-4,7,10-triazacyclononadec-15-ene-2,5,8,11-tetraone (**SI3**).* To a stirred solution of cyclization precursor **27** (12.5 mg, ca. 10.2 μmol) in CH_2Cl_2 (0.8 mL) at room temperature was added piperidine (0.2 mL). After stirring for 30 min, the reaction mixture was concentrated. To a stirred solution of the resulting amine in CH_2Cl_2 (1.8 mL) cooled at 0 °C were added HOBt (3.1 mg, 22.9 μmol) and EDCI·HCl (4.9 mg, 25.5 μmol) and then warmed to room temperature. After stirring for 8.5 h, the reaction mixture was diluted with 10% aqueous citric acid (20 mL) and extracted with EtOAc (40 mL). The organic layer was washed with saturated aqueous $NaHCO_3$ (20 mL) and brine (20 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by PLC (hexane−EtOAc 1:1) to give cyclization product **SI3** (6.3 mg, 75% in 3 steps) as a colorless oil: $[\alpha]_D{}^{23}$ + 25.9 (c 1.00, $CHCl_3$); IR (neat, cm^{-1}) 3298, 2928, 2856, 1739, 1645, 1533, 1496, 1471, 1456, 1409, 1375, 1256, 1188, 1093, 996, 855, 778, 756, 700; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30−7.22 (m, 5H), 7.25−7.12 (m, 2H), 6.75 (d, J = 8.8 Hz, 1H), 6.66 (d, J = 9.8 Hz, 1H, NH), 6.23 (d, J = 6.3 Hz, 1H, NH), 6.01 (ddt, J = 17.1, 10.5, 4.9 Hz, 1H), 5.51 (dd, J = 12.2, 4.9 Hz, 1H), 5.42 (dd, J = 17.1, 1.5 Hz, 1H), 5.27 (dd, J = 10.5, 1.5 Hz, 1H), 5.20 (d, J = 6.3 Hz, 1H), 5.00 (dd, J = 9.8, 6.3 Hz, 1H), 4.88 (t, J = 6.3 Hz, 1H), 4.82 (m, 1H), 4.61 (qd, J = 6.8, 6.3 Hz, 1H), 4.54 (d, J = 4.9 Hz, 2H), 3.39 (dd, J = 15.6, 4.9 Hz, 1H), 2.67 (dd, J = 15.6, 12.2 Hz, 1H), 2.55 (s, 3H), 2.34 (m, 1H), 2.05−1.96 (m, 3H), 1.90−1.83 (m, 1H), 1.77- 1.67 (m, 4H), 1.55−1.51 (m, 2H), 1.48 (s, 3H), 1.21 (d, J = 6.3 Hz, 3H), 0.91 (d, J = 6.8 Hz, 3H), 0.81 (s, 9H), 0.07 (s, 3H), −0.16 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.1, 173.6, 169.1, 168.6, 153.7, 140.4, 134.6, 133.2, 132.6, 130.8, 128.5, 128.3 (2C), 127.4 (2C), 125.0, 117.8, 113.7, 112.1, 77.3, 74.8, 71.3, 69.8, 58.5, 57.1, 45.6, 36.1, 35.0, 33.7, 32.2, 30.7, 27.5, 25.8 (3C), 24.0, 20.3, 18.1, 17.3, 15.6, −4.7, −4.9; HRMS (ESI) m/z 826.3460, calcd for $C_{42}H_{61}{}^{79}BrN_3O_7Si$ $[M + H]^+$ 826.3462.

*(3R,6R,9S,19S,E)-6-(4-(Allyloxy)-3-bromobenzyl)-3-((R)-hydroxy-(phenyl)methyl)-7,9,16,19-tetramethyl-1-oxa-4,7,10-triazacyclononadec-15-ene-2,5,8,11-tetraone (**3**).* To a stirred solution of cyclization product **SI3** (8.5 mg, 10.2 μmol) in THF (0.05 mL) at 0 °C were added acetic acid (0.01 mL, 170 μmol) and a 1 M solution of TBAF in THF (0.1 mL, 0.1 mmol). After stirring for 15 min, the reaction mixture was warmed to room temperature and stirred for 5 h, and then at 50 °C for 14 h. The reaction mixture was diluted with saturated NH_4Cl aq. (5 mL) and extracted with EtOAc (2 × 10 mL). The combined organic layers were washed with brine (10 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by PLC (hexane−EtOAc 2:3) to give Kazmaier's intermediate **3** (5.7 mg, 78%) as a colorless solid: $[\alpha]_D{}^{23}$ + 32.8 (c 1.00, $CHCl_3$); IR (neat, cm^{-1}) 3310, 2929, 2855, 1733, 1653, 1533, 1496, 1456, 1410, 1376, 1256, 1135, 1098, 1048, 995, 931, 881, 806, 753, 700; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 (d, J = 7.2 Hz, 2H), 7.32 (m, 2H), 7.28 (m, 1H), 7.24 (d, J = 2.2 Hz, 1H), 7.18 (d, J = 8.3 Hz, 1H, NH), 7.01 (dd, J = 8.5, 2.2 Hz, 1H), 6.74 (d, J = 8.5 Hz, 1H), 6.01 (m, 1H, NH), 6.00 (ddt, J = 17.3, 11.8, 5.2 Hz, 1H), 5.57 (dd, J = 11.8, 5.4 Hz, 1H), 5.43 (dq, J =

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17.3, 1.6 Hz, 1H), 5.29−5.25 (m,2H), 4.99 (dd, J = 8.3, 6.7 Hz, 1H), 4.93 (t, J = 6.4 Hz, 1H), 4.81 (m, 1H), 4.54 (dt, J = 5.2, 1.6 Hz, 2H), 4.40 (m, 1H), 3.39 (dd, J = 15.3, 5.4 Hz, 1H), 2.66 (dd, J = 15.3, 11.8 Hz, 1H), 2.41−2.34 (m, 4H), 2.20−1.84 (m, 6H), 1.73−1.55 (m, 3H), 1.51 (s, 3H), 1.24 (d, J = 6.3 Hz, 3H), 0.99 (d, J = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.4, 174.2, 170.5, 169.8, 153.6, 139.8, 134.0, 133.2, 132.6, 130.9, 128.5, 128.3 (2C), 128.1, 127.3 (2C), 125.5, 117.8, 113.6, 112.0, 74.3, 71.7, 69.8, 57.7, 56.4, 45.7, 35.8, 34.9, 33.0, 31.9, 30.2, 27.7, 23.7, 20.2, 16.4, 15.4; HRMS (ESI) m/z 712.2598, calcd for $C_{36}H_{47}{}^{79}BrN_3O_7$ $[M + H]^+$ 712.2597.

Miuraenamide A and D. To a stirred solution of ketone **28** (5.4 mg, 7.6 μmol) in MeOH (0.1 mL) cooled at 0 °C was added a solution of diazomethane in Et_2O (0.5 mL) (prepared by *N*-methyl-*N*-nitroso-*p*-toluenesulfonamide (130 mg, 0.607 mmol), Et_2O (10.2 mL), 85% KOH (1 g, 15 mmol), H_2O (1.6 mL), and EtOH (2 mL)). After stirring at 0 °C for 12 h, to the reaction mixture was added a solution of diazomethane in Et_2O (0.3 mL) at 0 °C. After stirring at 0 °C for 2 h, the reaction mixture was diluted with a 1 M solution of acetic acid in toluene and concentrated to give crude enol ether, which was then subjected to the allyl deprotection without further purification. To a stirred solution of the crude enol ether in dry MeOH (0.1 mL) at room temperature were added quinaldic acid (0.8 mg, 4.6 μmol) and Ru-catalyst $CpRu^{II}(MeCN)_3PF_6$ (0.2 mg, 0.5 μmol). After stirring for 1 h, the solvent was evaporated in vacuo, and the catalyst was removed by column chromatography (silica gel, hexane/EtOAc 1:3). The reaction was not completed, and then, the crude product was subjected to the allyl deprotection again. To a stirred solution of the crude product in 0.1 mL of dry MeOH at room temperature were added quinaldic acid (1.0 mg, 5.7 μmol) and Ru-catalyst $CpRu^{II}(MeCN)_3PF_6$ (0.5 mg, 1.2 μmol). After stirring for 2.5 h, the solvent was evaporated in vacuo, and the catalyst was removed by column chromatography (silica gel, hexane/EtOAc 1:3). The crude product was purified by HPLC (Nacalai AR-II (φ 20 × 250 mm), MeCN/H_2O 55:45, flow rate 5 mL/min, detection UV 215 nm) to give miuraenamide A (**1**) (2.6 mg, t_R = 34.8 min, 50%) and miuraenamide D (**2**) (1.2 mg, t_R = 48.6 min, 23%) as colorless solids.

Miuraenamide A (1). $[\alpha]_D^{23}$ + 45 (c 0.20, MeOH); IR (neat, cm^{-1}) 3334, 2934, 1635, 1508, 1446, 1362, 1335, 1254, 1215, 1116, 816, 754, 704, 665; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33 (m, 1H), 7.28 (d, J = 1.9 Hz, 1H), 7.23−7.18 (m, 4H), 7.23−7.18 (m, 1H, NH) 7.06 (s, 1H, NH), 6.96 (dd, J = 2.0, 8.3 Hz, 1H), 6.88, (d, J = 8.3 Hz, 1H), 5.49 (s, 1H, OH), 5.12−5.04 (m, 3H), 4.79 (qd, J = 6.8, 6.4 Hz, 1H), 3.48 (s, 3H), 3.24 (dd, J = 13.4, 10.5 Hz, 1H), 2.86 (s, 3H), 2.60 (dd, J = 13.4, 5.1 Hz, 1H), 2.33 (dt, J = 13.2, 8.3 Hz, 1H), 2.22−2.12 (m, 2H), 2.09−2.05 (m, 2H), 1.97 (m, 1H), 1.89−1.80 (m, 2H), 1.70 (m, 1H), 1.62 (m, 1H), 1.60 (s, 3H), 1.33 (d, J = 6.4 Hz, 3H) 1.29 (d, J = 6.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.5, 172.9, 168.9, 165.9, 165.1, 151.1, 134.8, 132.6, 131.8, 130.5, 130.2 (2C), 128.6 (2C), 128.3 (2C), 125.5, 116.1, 111.2, 110.1, 70.4, 58.7, 56.8, 46.2, 35.1, 35.0, 33.4, 31.9, 30.3, 26.6, 26.0, 19.8, 17.3, 16.4; HRMS (ESI) m/z 684.2278, calcd for $C_{34}H_{43}{}^{79}BrN_3O_7$ $[M + H]^+$ 684.2279.

Miuraenamide D (2). $[\alpha]_D^{23}$ −36 (c 0.14, MeOH); IR (neat, cm^{-1}) 3330, 2931, 1635, 1507, 1447, 1361, 1297, 1217, 1123, 755, 701; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44−7.37 (m, 4H), 7.34−7.28 (m, 3H), 7.25−7.23 (m, 1H), 7.12 (dd, J = 8.3, 1.7 Hz Hz, 1H), 6.94 (d, J = 8.4 Hz, 1H), 5.53−5.46 (m, 1H), 5.48 (s, 1H, OH), 4.93 (t, J = 6.9 Hz, 1H), 4.83 (dq, J = 7.8, 6.1 Hz, 1H), 4.61 (m, 1H), 3.41 (s, 3H), 3.35 (dd, J = 14.5, 8.4 Hz, 1H), 2.91 (s, 3H), 2.84 (dd, J = 14.5, 6.9 Hz, 1H), 2.24−2.17 (m, 1H), 2.14−2.07 (m, 1H), 2.03−1.96 (m, 2H), 1.90−1.82 (m, 2H), 1.70−1.60 (m, 2H), 1.52 (s, 3H), 1.37 (m, 1H), 1.33 (d. 6.3 Hz, 3H), 1.05 (m, 1H) 0.66 (d, 6.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.4 (2C), 168.0, 165.5 (2C), 151.2, 134.8, 132.8, 132.4, 130.7, 129.9, 129.6, 129.3 (2C), 128.2 (2C), 125.3, 116.2, 110.2, 107.8, 70.2, 57.5, 56.6, 46.2, 35.1, 34.5, 32.6, 31.7, 30.2, 26.2, 25.8, 18.7, 17.5, 16.3; HRMS (ESI) m/z 684.2264, calcd for $C_{34}H_{43}{}^{79}BrN_3O_7$ $[M + H]^+$ 684.2279.

(9H-Fluoren-9-yl)methyl ((4R,5S)-2,2-Dimethyl-4-phenyl-1,3-dioxan-5-yl)carbamate (18). To a stirred solution of diol **15** (10.2 mg, 26.1 μmol) in 2,2-dimethoxypropane (0.5 mL) at room temperature was added *p*-TsOH·H_2O (0.8 mg, 4 μmol). After stirring 2.5 h, the reaction mixture was diluted with saturated aqueous $NaHCO_3$ (5 mL) and extracted with EtOAc (3 × 5 mL). The combined extracts were washed with brine (15 mL), dried (Na_2SO_4), and concentrated. The residual oil was purified by PLC (hexane−EtOAc 5:4) to give acetal **18** (7.6 mg, 68%) as a colorless oil: $[\alpha]_D^{23}$ + 18.9 (c 0.506, $CHCl_3$); IR (neat, cm^{-1}); 3411, 3309, 3065, 3039, 2996, 2926, 2855, 1700, 1542, 1451, 1382, 1294, 1028, 759; ^{1}H NMR (400 MHz, C_6D_6) δ 7.58 (d, J = 7.3 Hz, 2H), 7.31 (t, J = 7.3 Hz, 2H), 7.26−7.05 (m, 9H), 4.56 (d, J = 9.8 Hz, 1H), 4.35 (dd, J = 10.7, 5.9 Hz, 1H), 4.23 (dd, J = 10.7, 5.4 Hz, 1H), 3.82 (m, 1H), 3.75 (dd, J = 11.0, 5.4 Hz, 1H), 3.67 (dd, J = 11.0, 10.3 Hz, 1H), 3.61 (m, 1H, NH), 3.56 (m, 1H), 1.52 (s, 3H), 1.39 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.5, 143.8, 143.7, 141.4 (2C), 138.7, 128.7 (4C), 127.8 (2C), 127.4 (2C), 127.1 (2C), 125.0 (2C), 120.1 (2C), 99.4, 74.7, 66.7, 63.1, 51.5, 47.2, 28.9, 19.5; HRMS (ESI) m/z 430.2017, calcd for $C_{27}H_{28}NO_4$ $[M + H]^+$ 430.2018.

Anti-*Phytophthora* Activity. The phytopathogenic oomycete *Phytophthora capsici* NBRC 30696, purchased from a supplier, was cultured on a potato-agar medium [20 mL; potato broth from 200 g of fresh potato, glucose (20 g), and agar (20 g) in 1 L] in a 9 cm dish at 25 °C for 7 days in the dark. A piece of the colony was then inoculated on the center of a 5% V8-agar medium [20 mL; V8 vegetable juice (5 mL), agar (2 g), and water (95 mL) in 100 mL] in a 9 cm dish and incubated at 25 °C and 60% humidity for 48 h in the dark until the colony grew to 3−4 cm in diameter. A DMSO solution (5 μL) of a sample was impregnated into a paper disc (for antibiotic assay, thin 6 mm size), and the disk was placed 1 cm away from the front of the colony. After incubating for 22−24 h, the distance between the edge of the colony and the paper disc (control: 0 mm) was measured.

Cytotoxicity Assay for the HeLa-S3 Cell Line. HeLa-S3 (SC) cells were purchased from suppliers. The cells were cultured in Eagle's minimal essential medium (EMEM) supplemented with 10% bovine serum, 100 units/mL penicillin, and 100 μg/mL streptomycin. A total of 10,000 cultured cells were seeded into each well of a 96-well plate containing 99 μL of the same medium. After preincubation for 24 h at 37 °C in an atmosphere of 5% CO_2, a compound in 1 μL of dimethyl sulfoxide (DMSO) was added to each well, and the cells were incubated for an additional 48 h. A solution (10 μL) of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) in phosphate buffered saline (PBS) (5 mg/mL) was then added to each well, and the plate was incubated for an additional 3 h. The medium was removed by aspiration, generated formazan was dissolved in 100 μL of DMSO, and the absorbance was measured at 595 nm using a Multiskan FC microplate reader.

Cytotoxicity Assay for HeLa cell line. HeLa cells were cultured at 37 °C with 5% CO_2 in DMEM supplemented with 10% heat-inactivated FBS, 100 units/mL penicillin, 100 μg/mL streptomycin, 0.25 μg/mL amphotericin, 300 μg/mL L-glutamine, and 2.25 mg/mL $NaHCO_3$. HL60 cells were cultured at 37 °C with 5% CO_2 in RPMI supplemented with 10% heat-inactivated FBS, 100 units/mL penicillin, 100 μg/mL streptomycin, 0.25 μg/mL amphotericin, 300 μg/mL L-glutamine, and 2.25 mg/mL $NaHCO_3$. HeLa cells were seeded at 2 × 10^4 cells/well in 96-well plates and cultured overnight. HL60 cells were seeded at 1 × 10^5 cells/well in 96-well plates. Various concentrations (0.1 nM, 1 nM, 10 nM, and 100 nM) of compounds were then added, and cells were incubated for 72 h. Cell proliferation was measured by the MTT assay.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.6b02061.

Spectroscopic data and ^{1}H and ^{13}C NMR spectra (PDF)

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Notes

The authors declare no competing financial interest.

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(15) (a) Boden, E. P.; Keck, G. E. *J. Org. Chem.* **1985**, *50*, 2394−2395. (b) This condensation hardly proceeded with HATU, and the use of excess DMAP only resulted in epimerization at the α position of carboxylic acid. To suppress side reactions (formation of *N*-acylurea and/or removal of the Fmoc group), we used HCl·py as a proton source..
(16) The anti-*Phytophthora* activity of natural **2** was about 30 times stronger than our previous report.[2] In the previous assay, the accuracy of its concentration could not be enough because of the scarcity of **2**. Photographs of the anti-*Phytophthora* activity of synthesized **1** and **2** and natural products are shown in Supporting Information, p S45.

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