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# Total Synthesis and Antimicrobial Activity of Chlorocatechelin A

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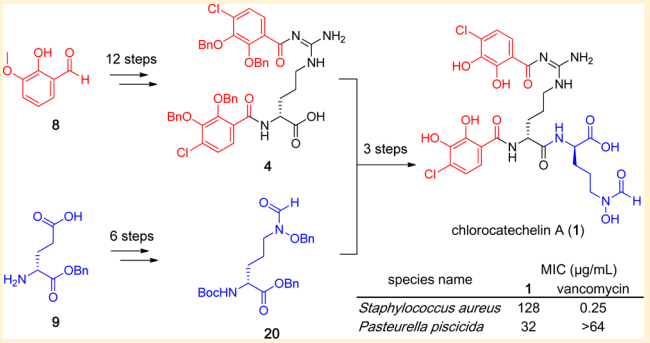
Supporting Information

**ABSTRACT:** Chlorocatechelin A (**1**) is a structurally unique microbial siderophore containing two units of 4-chloro-2,3-dihydroxybenzoic acid (CDB) and a characteristic acylguanidine structure. Purification from the microbe culture is not an easy task due to the lability of the acylguanidine and its chelating nature. Here we report the first convergent total synthesis and antimicrobial activity of chlorocatechelin A (**1**). The bis-acylated arginine was constructed using a Schotten–Baumann reaction whereas the CDB component was synthesized from *o*-vanillin (**8**). Condensation with an ornithine derivative synthesized from 1-benzyl D-glutamate was followed by deprotection in basic and neutral conditions to complete the total synthesis. We examined the antimicrobial activity of chlorocatechelin A (**1**) and found that this siderophore was active against desferrioxamine B (DFB)-sensitive microbes including the fish pathogen *Pasteurella piscicida*.




## INTRODUCTION

Iron is essential for organisms, as it plays important roles in primary and secondary metabolisms. Microbes and plants have to take up necessary iron from surrounding environments, where most iron exists as oxidized, insoluble Fe(III). To overcome this bioavailability issue they biosynthesize and excrete low molecular weight compounds called siderophores.[1] A typical siderophore possesses three bidentate groups by which Fe(III) atom is solubilized through forming a stable, soluble octahedral Fe(III)–siderophore complex. The bidentate groups are usually catecholate, hydroxamate, or α-hydroxycarboxylate; some siderophores possess three catecholate groups (e.g., enterobactin[2,3]), some possess three hydroxamate groups (e.g., desferrioxamine B (DFB)[2,4]), and others include a mixture of two or three bidentate types in one molecule.[2] On the other hand, the backbone structures to which bidentate groups are attached vary among siderophores.

Recently, we screened for siderophores from microbial extracts and isolated two novel siderophores named chlorocatechelins A (**1**) and B (**2**) from the culture extract of *Streptomyces* sp. ML93-86F2 (Figure 1). Their structures were determined by spectroscopic analyses and degradation study.[5] Chlorocatechelin A (**1**) represents a characteristic structure; it contains two units of 4-chloro-2,3-dihydroxybenzoic acid (CDB), which has never been reported in natural products, and one unit of CDB is condensed with guanidine to form an acylguanidine. The acylguanidine is rarely found in natural products;[6−8] rare examples are *Rhodococcus*-derived siderophores, heterobactin A (**3**), and its derivatives.[9,10] This acylguanidine structure was important for the Fe(III)-chelating activity of **1**, as it decomposed in acidic conditions to furnish a lower-affinity siderophore **2**.[5] The biological activity of this unique metabolite was of interest to us; however, we had difficulty in obtaining highly purified **1** from microbial extracts due to its lability in acidic solutions and its metal-chelating

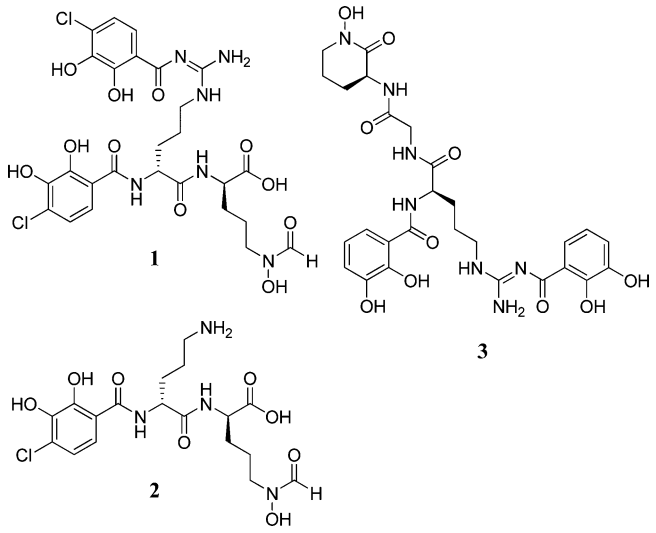



**Figure 1.** Chemical structures of chlorocatechelins A (**1**) and B (**2**), and heterobactin A (**3**).

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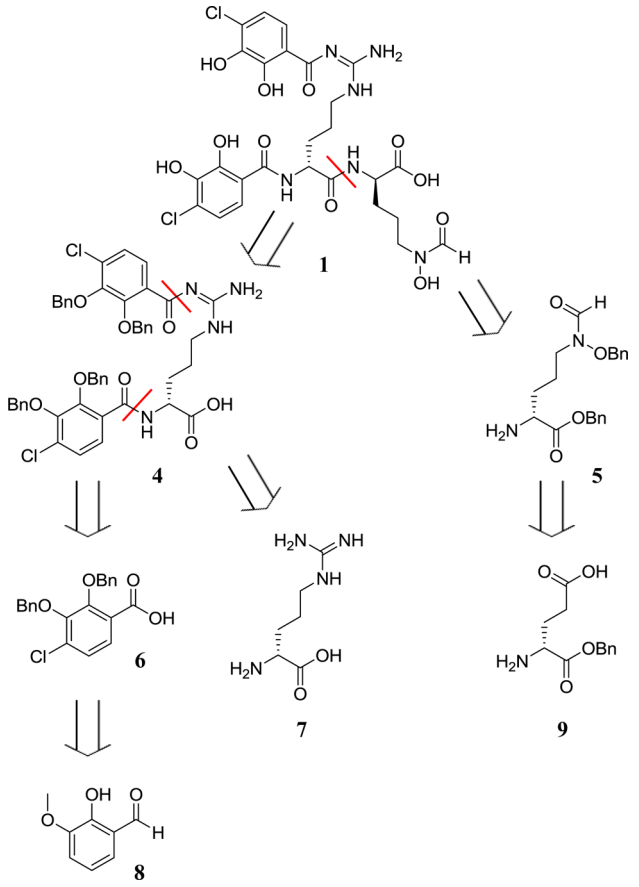
nature. To obtain a sufficient amount of pure **1** for biological evaluation, we conducted a convergent total synthesis of **1**. Using synthesized **1** and natural **2**, we investigated antimicrobial activities of chlorocatechelins.

## RESULTS AND DISCUSSION

**Total Synthesis of Chlorocatechelin A (1).** The backbone structure of chlorocatechelin A (**1**) is a dipeptide, to which two units of CDB are condensed. We planned to synthesize **1** by conjugating the left segment **4** and the right segment **5** (Scheme 1). In the previous study, we noticed that

**Scheme 1. Retrosynthetic Analysis of Chlorocatechelin A (1)**



compound **1** decomposed under acidic conditions; hence, protecting groups needed to be cleaved under neutral or basic conditions.[5] We chose benzyl groups for protecting all of the hydroxyl groups. The left segment **4** could be obtained by conjugating two molecules of benzyl-protected CDB **6** and one molecule of D-Arg (**7**). Para-selective chlorination was expected to be achieved by choosing *o*-vanillin **8** as a starting material. The right segment **5** could be synthesized from a D-glutamate derivative **9** through conversion of the functional groups.

Synthesis of the left segment **4** was commenced from *o*-vanillin **8** (Scheme 2). Compound **8** was first protected with an acetyl group followed by nitration to obtain C4-substituted compound **11**, according to the procedure reported by Morie and co-workers.[11] Oxidation of the aldehyde and reduction of the nitro group to an amino group proceeded quantitatively, and subsequent Sandmeyer reaction gave 4-chloro-2-hydroxy-3-methoxybenzoic acid **14**. To obtain benzyl-protected CDB **6**, compound **14** was demethylated with the Lewis acid $BBr_3$, reacted with BnBr, and hydrolyzed in basic conditions. These procedures furnished protected CDB **6** in a good yield. The carboxylic acid **6** was converted to acid chloride, followed by reaction with D-Arg under Schotten–Baumann conditions, yielding the left fragment **4** (28%) with recovered starting material **6** (70%). A few model experiments using benzoic acid as a substrate were tested and fixed this condition, though the conversion rate was low.

The protected right segment **20** was synthesized from 1-benzyl D-glutamate **9** in six steps (Scheme 3). First, the amino group in compound **9** was protected with a Boc group. The carboxylic acid in **16** was converted to acid anhydride and selectively reduced with $NaBH_4$ to obtain an alcohol **17**. The alcohol **17** was then subjected to Mitsunobu reaction with protected hydroxylamine to yield compound **18**. Deprotection of compound **18** by removal of the Ns group followed by formylation furnished compound **20**.

Compound **20** was deprotected by removal of the Boc group to yield the right segment **5**, followed by condensation with the left segment **4** (Scheme 4). Although active ester of the carboxylic acid **4** immediately gave an intramolecular condensation product, we could avoid this undesired reaction by mixing **4** and **5** in a minimum amount of the solvent before addition of condensation reagents. The final task was removal of the six benzyl groups. We first tried to deprotect all of them from compound **21** under a hydrogen atmosphere. However, this procedure furnished not only compound **1** but also several dechlorinated compounds mainly due to the low reactivity of the benzyl ester. Therefore, the ester was first cleaved in alkaline conditions to furnish compound **22**, and then the benzyl ethers were deprotected by hydrogenolysis to give compound **1**. The $^{1}H$ and $^{13}C$ NMR spectra and other physicochemical properties of synthesized **1** were identical with those of natural **1** (Supporting Information Figures S33 and S34).

**Antimicrobial Activities of Chlorocatechelins A (1) and B (2).** With a sufficient supply of pure **1** secured, we next investigated the biological effects of **1** and its degraded derivative **2** on microbes. Antimicrobial assays of synthesized **1**, natural **2**, and vancomycin (VCM) were conducted against DFB-sensitive microbes (Table 1) and DFB-insensitive ones (Table 2). VCM was much more effective against DFB-insensitive microbes though DFB-sensitive microbes were tolerant to VCM. Both **1** and **2** inhibited the growth of DFB-sensitive microbial strains including the fish pathogen *Pasteurella piscicida* at concentrations lower than those of DFB-insensitive ones. In addition, the high-affinity siderophore **1** showed activities against DFB-sensitive strains more potent than those of the low-affinity siderophore **2** and DFB. The Fe(III)-binding affinity of **1** is higher than those of **2** and DFB in cyclic voltammetry experiments,[5] indicating that **1** and **2** exhibited antimicrobial activities by limiting iron availability like that of DFB.[12]

## CONCLUSION

In this study, we convergently synthesized chlorocatechelin A (**1**) from *o*-vanillin (**8**) in 15 steps. This first total synthesis of an acylguanidine-containing siderophore confirmed the unique structure of **1**, which we previously determined by spectroscopic analysis and degradation studies.[5] Considering the fact that the structure of heterobactin A (**3**) was first misassigned in 2001[9] and revised in 2013,[10] structure elucidation of acylguanidine-containing siderophores seems to have some



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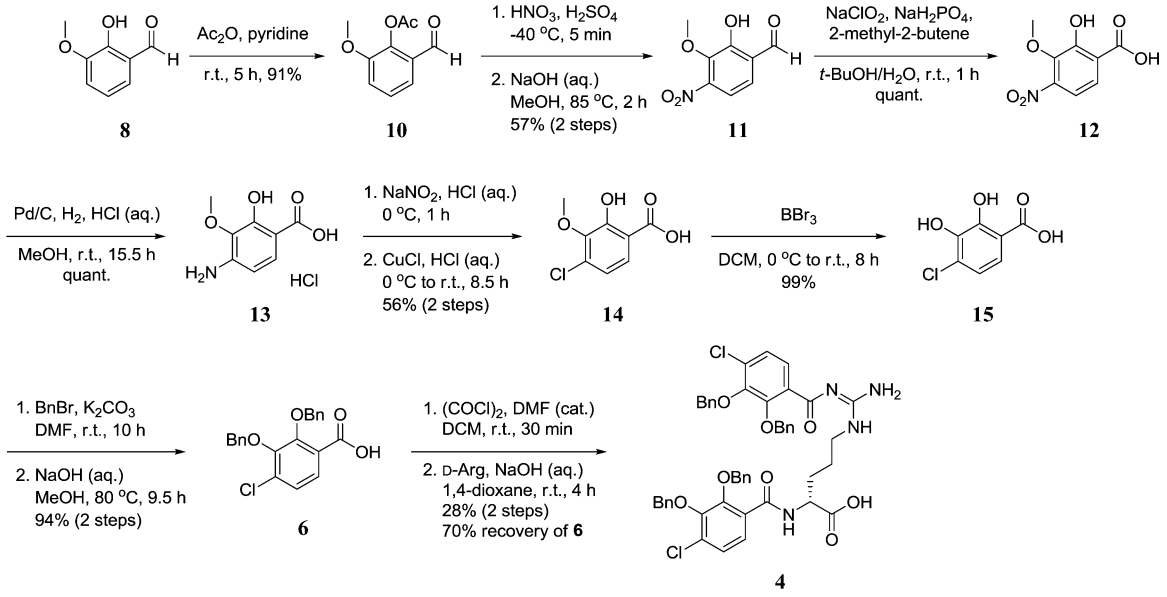
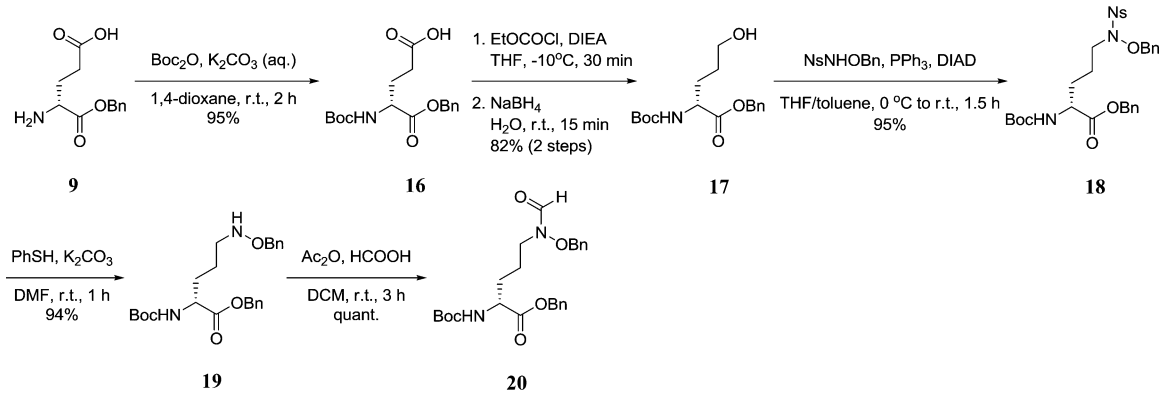
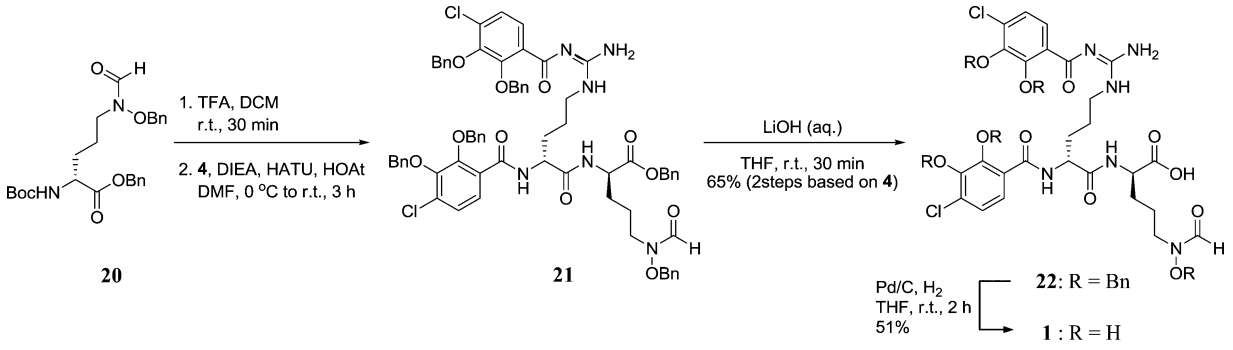
**Scheme 2. Synthesis of the Left Segment 4**




**Scheme 3. Synthesis of the Protected Right Segment 20**




**Scheme 4. Synthesis of Chlorocatechelin A (1)**




difficulties; this is partly due to the poverty of 2D NMR information around acylguanidines.[5] Our synthetic route is concise, which would be applicable to the synthesis and the confirmation of the structures of other acylguanidine-containing siderophores. Using synthesized chlorocatechelin A (**1**) and natural chlorocatechelin B (**2**), we revealed that antimicrobial activity and iron affinity of chlorocatechelins had good correlations. Chlorocatechelin A (**1**), a higher affinity siderophore, exhibited more potent antimicrobial activity, indicating that chlorocatechelins show antimicrobial activity by limiting iron availability like DFB. Biosynthetic mechanisms and environmental impact of chlorocatechelins are the next issues to be explored.

## EXPERIMENTAL SECTION

**General Methods.** All reagents and solvents were used as received from commercial suppliers. IR spectra were measured using an FTIR spectrometer equipped with a ZnSe ATR plate. Optical rotations were determined using the sodium D line (589 nm). NMR spectra were measured on a 500 MHz instrument. $^{1}H$ and $^{13}C$ chemical shifts ($\delta$)



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**Table 1. Antimicrobial Activities of 1, 2, and VCM against DFB-Sensitive Microbes**

| species name | strain | MIC (μg/mL) 1 | 2 | VCM |
|---|---|---|---|---|
| *Staphylococcus aureus* | FDA 209P[a] | 128 | 128 | 0.25 |
| *Pasteurella piscicida* | 6395[b] | 32 | 64 | >64 |
| | P-3340[b] | 32 | 64 | >64 |
| | P-3343[b] | 32 | 64 | >64 |
| | P-3344[b] | 32 | 64 | >64 |
| | P-3346[b] | 32 | 64 | >64 |
| | P-3347[b] | 32 | 64 | >64 |
| | P-3349[b] | 32 | 64 | >64 |
| | P-3350[b] | 32 | 64 | >64 |
| | P-3353[b] | 32 | 64 | >64 |
| | P-3179[b] | 32 | 64 | >64 |
| *Mannheimia hemolytica* | N811 BBP 0101[a] | 32 | 64 | >64 |
| *Escherichia coli* | K-12[a] | 32 | 64 | 32 |

[a]MICs of DFB against *S. aureus*, *M. hemolytica*, and *E. coli* were 128, 64, and 64 μg/mL, respectively. [b]DFB partially inhibited the growth of these *P. piscicida* strains at 64 μg/mL, whereas the MICs were >64 μg/mL.

**Table 2. Antimicrobial Activities of 1, 2, and VCM against DFB-Insensitive Microbes**

| species name | strain | MIC (μg/mL) 1 | 2 | VCM |
|---|---|---|---|---|
| *Staphylococcus aureus* | FDA 209P | >64 | >64 | 0.125 |
| | Smith | >64 | >64 | 0.25 |
| | MS9610 | 128 | 128 | 0.25 |
| | MRSA No.5 | 128 | 128 | 0.25 |
| | MRSA No.17 | 128 | 128 | 0.25 |
| | MS16526 | 128 | 128 | 0.25 |
| | TY-04282 | 128 | 128 | 0.25 |
| | Mu50 | 128 | 128 | 0.5 |
| *Micrococcus luteus* | FDA 16 | 128 | 128 | 0.0625 |
| | IFO 3333 | 128 | 128 | 0.125 |
| | PCI 1001 | 128 | 128 | 0.125 |
| *Bacillus subtilis* | NRRL B-558 | 128 | >128 | 0.0625 |
| | PCI 219 | 128 | >128 | 0.0625 |
| *Bacillus cereus* | ATCC10702 | 128 | >64 | 0.125 |
| *Corynebacterium bovis* | 1810 | >64 | >64 | 0.0625 |
| *Enterococcus faecalis* | JCM 5803 | >64 | >64 | 0.25 |
| | NCTC12201 | >64 | >64 | 128 |
| | NCTC12203 | >64 | >64 | 128 |
| *Enterococcus faecium* | JCM 5804 | >64 | >64 | 0.125 |
| | NCTC12202 | >64 | >64 | 128 |
| | NCTC12204 | >64 | >64 | 128 |
| *Escherichia coli* | NIHJ | >64 | >64 | 16 |
| | K-12 | >64 | >64 | 8 |
| | K-12 ML1629 | >64 | >64 | 16 |
| | BEM11 | >64 | >64 | 16 |
| | BE1121 | >64 | >64 | 1 |
| | BE1186 | >64 | >64 | 32 |
| *Shigella dysenteriae* | JS11910 | 64 | 64 | 16 |
| *Salmonella enteritidis* | 1891 | >64 | >64 | 32 |
| *Proteus vulgaris* | OX19 | 64 | 64 | 2 |
| *Proteus mirabilis* | IFM OM-9 | 64 | 64 | 1 |
| *Serratia marcescens* | B-0524 | >64 | >64 | 16 |
| *Pseudomonus aeruginosa* | A3 | 64 | >64 | 16 |
| *Klebsiella pneumoniae* | PCI 602 | >64 | >64 | 64 |
| *Candida albicans* | 3147 | 64 | 64 | 128 |

are relative to the solvent: $\delta_H$ 3.31 and $\delta_C$ 49.00 for $CD_3OD$ ($CD_3OH$), $\delta_H$ 2.50 and $\delta_C$ 39.52 for DMSO-$d_6$, $\delta_H$ 2.05 and $\delta_C$ 29.84 for acetone-$d_6$, and $\delta_H$ 7.26 and $\delta_C$ 77.16 for $CDCl_3$. Mass spectral data were collected with ESI-TOF-MS.

**Synthesis of Left Segment 4.** *2-Formyl-6-methoxyphenyl Acetate (**10**).* To a stirred solution of 2-hydroxy-3-methoxybenzaldehyde (**8**; 9.82 g, 64.5 mmol) in dry pyridine (10 mL) was added $Ac_2O$ (6.7 mL, 71 mmol). After being stirred for 5 h at rt, the reaction mixture was poured into ice cold 6 N aq HCl (60 mL) to form precipitate. The precipitate was washed with 1 N aq HCl and $H_2O$ to give **10** (11.68 g, 60.15 mmol, 93.3%) as a colorless solid: $^1$H NMR ($CDCl_3$, 500 MHz) $\delta$ 10.09 (s, 1H), 7.41 (dd, $J$ = 8.0, 1.5 Hz, 1H), 7.28 (dd, $J$ = 8.0, 8.0 Hz, 1H), 7.18 (dd, $J$ = 8.0, 1.5 Hz, 1H), 3.82 (s, 3H), 2.36 (s, 3H); $^{13}$C NMR ($CDCl_3$, 125 MHz) $\delta$ 188.7, 168.7, 151.8, 141.5, 129.2, 126.8, 121.2, 117.9, 56.3, 20.4; HRMS (ESI) $m/z$ 193.0506 $[M - H]^-$ calcd for $C_{10}H_9O_4$, 193.0506; mp 65 °C. Spectral data were in agreement with those reported previously.[13]

*2-Hydroxy-3-methoxy-4-nitrobenzaldehyde (**11**).* According to the method of Morie et al.,[11] we added finely powdered compound **10** (2.51 g, 12.9 mmol) portionwise to a stirred solution of fuming $HNO_3$ (7.5 mL) and concd $H_2SO_4$ (1 mL) at −40 °C. After being stirred for 5 min at the same temperature, the mixture was poured into ice cold $H_2O$ (120 mL) and extracted with $CHCl_3$ (2 × 100 mL). The combined organic layers were washed with $H_2O$, sat. aq $NaHCO_3$ and brine, and dried over anhydrous $Na_2SO_4$. After concentration in vacuo, the residue was chromatographed ($SiO_2$, *n*-hexane/EtOAc = 4:1 to 3:1) to yield a mixture of **11** and acetylated form of **11**. This material was hydrolyzed in a mixture of MeOH (25 mL) and 2 M aq NaOH (10 mL) by refluxing for 1.5 h and concentrated in vacuo. The residue was dissolved in DCM (6 mL) and 1.5 N aq HCl (20 mL), which was stirred for 10 h and separated. The organic layer was washed with $H_2O$ (6 mL), dried over anhydrous $Na_2SO_4$ and concentrated in vacuo to give **11** (1.44 g, 7.30 mmol, 56.6%) as a yellow solid: $^1$H NMR ($CDCl_3$, 500 MHz) $\delta$ 11.39 (s, 1H), 9.98 (s, 1H), 7.43 (d, $J$ = 8.6 Hz, 1H), 7.29 (d, $J$ = 8.6 Hz, 1H), 4.07 (s, 3H); $^{13}$C NMR ($CDCl_3$, 125 MHz) $\delta$ 196.3, 156.6, 148.6, 141.9, 127.9, 123.0, 114.4, 62.2; HRMS (ESI) $m/z$ 196.0252 $[M - H]^-$ calcd for $C_8H_6NO_5$, 196.0251; mp 91 °C. Spectral data were in agreement with those reported previously.[11]

*2-Hydroxy-3-methoxy-4-nitrobenzoic Acid (**12**).* To a stirred solution of **11** (1.23 g, 6.24 mmol) and 2-methyl-2-butene (6.2 mL) in 18.5 mL of *t*-BuOH was added dropwise 80% $NaClO_2$ (845 mg, 7.47 mmol) in 6.2 mL of 1 M aq $NaH_2PO_4$. After being stirred at rt for 20 min, the mixture was concentrated in vacuo and dissolved in 40 mL of EtOAc. The organic layer was washed with 20 mL each of 1 N aq HCl, $H_2O$ and brine, dried over anhydrous $Na_2SO_4$ and concentrated in vacuo to yield **12** (1.33 g, 6.24 mmol, 100%) as a light yellow solid: $^1$H NMR (acetone-$d_6$, 500 MHz) $\delta$ 10.85 (br s, 1H), 7.83 (d, $J$ = 8.6 Hz, 1H), 7.30 (d, $J$ = 8.6 Hz, 1H), 4.01 (s, 3H); $^{13}$C NMR (acetone-$d_6$, 125 MHz) $\delta$ 171.8, 157.9, 149.2, 141.9, 126.2, 117.3, 113.8, 62.1; HRMS (ESI) $m/z$ 212.0200 $[M - H]^-$ calcd for $C_8H_6NO_6$, 212.0201; mp 208 °C.

*4-Carboxy-3-hydroxy-2-methoxybenzenaminium Chloride (**13**).* To a solution of **12** (1.05 g, 4.93 mmol) in a mixed solvent of MeOH (20 mL) and 1 N aq HCl (7 mL) was added 10% Pd/C (53.1 mg), and the mixture was stirred under hydrogen. After being stirred for 15.5 h, the catalyst was removed with Celite and the filtrate was concentrated in vacuo to give **13** (1.09 g, 4.96 mmol, 100%) as a brown amorphous solid: IR (neat) 3346, 3000 (br), 2942, 2593, 1629, 1579, 1508, 1474, 1385, 1307, 1260 cm$^{-1}$; $^1$H NMR ($CD_3OD$, 500 MHz) $\delta$ 7.70 (d, $J$ = 8.7 Hz, 1H), 6.89 (d, $J$ = 8.7 Hz, 1H), 4.05 (s, 3H); $^{13}$C NMR ($CD_3OD$, 125 MHz) $\delta$ 173.0, 156.9, 142.2, 131.3, 126.7, 115.0, 113.7, 61.5; HRMS (ESI) $m/z$ 182.0459 $[M - H]^-$ calcd for $C_8H_8NO_4$, 182.0459.

*4-Chloro-2-hydroxy-3-methoxybenzoic Acid (**14**).* To a stirred solution of **13** (1.86 g, 8.47 mmol) in 68 mL of 1 N aq HCl was added $NaNO_2$ (910 mg, 13.2 mmol) in $H_2O$ (2.5 mL) at 0 °C. The mixture was stirred for 1 h at the same temperature, to which CuCl (2.57 g, 26.0 mmol) in 1 N aq HCl (3.5 mL) was added. The reaction mixture was warmed slowly to rt. After being stirred for 5 h, the reaction



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mixture was extracted with $Et_2O$ (3 × 30 mL), and the combined organic layers were washed with brine, dried over anhydrous $Na_2SO_4$, and concentrated in vacuo. The residue was chromatographed ($SiO_2$, *n*-hexane/acetone/acetic acid = 80:20:1) to give **14** (965 mg, 4.76 mmol, 56.2%) as a brown solid: IR (neat) 3003 (br), 2947, 2856, 2596, 2534, 1654, 1599, 1458, 1426, 1389, 1306, 1229, 1170 $cm^{-1}$; $^1H$ NMR ($CD_3OD$, 500 MHz) $\delta$ 7.54 (d, $J$ = 8.6 Hz, 1H), 6.87 (d, $J$ = 8.6 Hz, 1H), 3.86 (s, 3H); $^{13}C$ NMR ($CD_3OD$, 125 MHz) $\delta$ 173.1, 157.6, 145.8, 134.9, 126.6, 120.6, 114.1, 60.8; HRMS (ESI) $m/z$ 200.9964 $[M - H]^-$ calcd for $C_8H_6ClO_4$, 200.9960; mp 173 °C.

*4-Chloro-2,3-dihydroxybenzoic Acid (**15**).* A stirred solution of **14** (1.61 g, 7.95 mmol) in 14.6 mL of dry DCM under nitrogen atmosphere was cooled to 0 °C, to which 1 M $BBr_3$ in DCM (17.5 mL, 17.5 mmol) was added. After being stirred for 6 h at rt, the reaction was quenched with 10 M aq NaOH (6 mL), acidified with 10 N aq HCl (3 mL), and extracted with EtOAc (2 × 100 mL). The combined organic layers were washed with $H_2O$ and brine, dried over anhydrous $Na_2SO_4$, and concentrated in vacuo to give **15** (1.48 g, 7.85 mmol, 98.7%) as a brown amorphous solid: IR (neat) 3605, 3510, 3010 (br), 2860, 2709, 2582, 1659, 1613, 1456, 1431, 1297, 1227, 1163 $cm^{-1}$; $^1H$ NMR (acetone-$d_6$, 500 MHz) $\delta$ 11.43 (br s, 1H), 8.56 (br s, 1H), 7.38 (d, $J$ = 8.7 Hz, 1H), 6.93 (d, $J$ = 8.7 Hz, 1H); $^{13}C$ NMR (acetone-$d_6$, 125 MHz) $\delta$ 172.6, 152.1, 143.4, 126.5, 121.3, 120.6, 112.0; HRMS (ESI) $m/z$ 186.9808 $[M - H]^-$ calcd for $C_7H_4ClO_4$, 186.9804.

*2,3-Bis(benzyloxy)-4-chlorobenzoic Acid (**6**).* To a stirred solution of **15** (1.43 g, 7.58 mmol) in dry DMF (7.5 mL) were added $K_2CO_3$ (3.25 g, 23.5 mmol) and BnBr (2.80 mL, 23.6 mmol). After being stirred for 10 h, the reaction was quenched with $H_2O$ and brine and extracted with EtOAc (2 × 100 mL). The combined organic layers were washed with sat. aq $NaHCO_3$, sat. aq $NH_4Cl$, and brine, dried over anhydrous $Na_2SO_4$, and concentrated in vacuo. The residue was chromatographed ($SiO_2$, *n*-hexane/EtOAc = 10:1) to give fractions containing benzyl ester. This mixture was dissolved in MeOH (15 mL) and 2 M aq NaOH (10 mL) and heated to 80 °C. After being stirred for 9.5 h at the same temperature, this mixture was evaporated to remove MeOH. The mixture was diluted in $H_2O$, washed with $Et_2O$ (2 × 10 mL), and acidified with aq HCl to form white precipitate. The precipitate was washed with $H_2O$ to give **6** (2.62 g, 7.11 mmol, 93.8%) as a white solid: IR (neat) 3032, 2900 (br), 2889, 2662, 2575, 1690, 1578, 1475, 1370, 1301, 1241 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 500 MHz) $\delta$ 10.92 (br s, 1H), 7.84 (d, $J$ = 8.8 Hz, 1H), 7.50 (dd, $J$ = 7.8, 2.3 Hz, 2H), 7.45−7.29 (m, 9H), 5.28 (s, 2H), 5.11 (s, 2H); $^{13}C$ NMR ($CDCl_3$, 125 MHz) $\delta$ 164.9, 152.4, 148.6, 136.0, 135.4, 134.4, 129.6, 129.5 (2C), 129.1 (2C), 128.9, 128.9 (2C), 128.8 (2C), 128.2, 126.5, 122.5, 78.1, 76.1; HRMS (ESI) $m/z$ 367.0743 $[M - H]^-$ calcd for $C_{21}H_{16}ClO_4$, 367.0743; mp 148 °C.

*(R)-2-(2,3-Bis(benzyloxy-4-chlorobenzamido)-5-(3-(2,3-bis-(benzyloxy-4-chlorobenzoyl)guanidine)pentanoic Acid (**4**).* To a stirred solution of **6** (2.52 g, 6.84 mmol) in dry DCM (28 mL) were added oxalyl chloride (600 μL, 7.00 mmol) and a catalytic amount of dry DMF. The mixture was stirred for 30 min and concentrated in vacuo. The residue was dissolved in 1,4-dioxane (18 mL) and added dropwise to a stirred solution of D-Arg (556 mg, 3.19 mmol) in 2.5 M aq NaOH (20 mL). After being stirred for 4 h, the reaction mixture was acidified with 10 N aq HCl (5.1 mL) and extracted with EtOAc (2 × 20 mL). The combined organic layers were washed with $H_2O$ and brine, dried over anhydrous $Na_2SO_4$, and concentrated in vacuo. The residue was chromatographed ($SiO_2$, $CHCl_3$/acetone/acetic acid = 50:50:1 and $CHCl_3$/MeOH = 20:1) to recover **6** (1.78 g, 4.83 mmol, 71%) and to yield **4** (833 mg, 0.951 mmol, 27.8%) as a colorless amorphous solid: $[\alpha]^{20}_D$ +3.4 (*c* 0.26, MeOH); IR (neat) 3317 (br), 3064, 3032, 2941, 1689, 1648, 1580, 1509, 1498, 1456, 1429, 1367, 1295, 1230 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$, 500 MHz) $\delta$ 9.30−8.40 (br m, 2H), 7.50−7.10 (m, 25H), 5.20−4.90 (m, 8H), 4.45−4.35 (m, 1H), 3.23−3.10 (m, 2H), 1.90−1.40 (m, 4H); $^{13}C$ NMR (DMSO-$d_6$, 125 MHz) $\delta$ 173.8 (2C), 164.3, 158.0, 151.0, 150.7, 148.3, 148.1, 136.8, 136.5, 136.3, 136.1, 130.3, 129.2, 129.0, 128.5−127.8 (21C), 125.5, 125.1, 124.8, 124.7, 76.0, 75.7, 75.0, 74.9, 52.5, 40.3, 28.6, 24.9; HRMS (ESI) $m/z$ 875.2626 $[M + H]^+$ calcd for $C_{48}H_{45}Cl_2N_4O_8$, 875.2609.

**Synthesis of Right Segment 5.** *(R)-5-(Benzyloxy)-4-(tert-butoxycarbonylamino)-5-oxopentanoic Acid (**16**).* To a stirred solution of 1-benzyl D-glutamate (**9**; 2.00 g, 8.42 mmol) in a mixed solvent of 1 M aq $K_2CO_3$ (8.42 mL) and 1,4-dioxane (8.4 mL) was added $Boc_2O$ (2.13 mL, 9.36 mmol), and it was stirred for 2 h at rt. The reaction mixture was cooled to 0 °C and quenched with 1 N aq HCl (16.8 mL), and aqueous layer was extracted with EtOAc (20 mL). Combined organic layers were washed with $H_2O$ (40 mL) and brine (25 mL), dried over anhydrous $Na_2SO_4$, and concentrated in vacuo. The residue was chromatographed ($SiO_2$, *n*-hexane/acetone = 1:4) to afford **16** (2.71 g, 8.03 mmol, 95.3%) as a white solid: $[\alpha]^{20}_D$ +30.3 (*c* 0.46, MeOH); $^1H$ NMR (acetone-$d_6$, 500 MHz) $\delta$ 10.6 (br s, 1H), 7.44−7.29 (m, 5H), 6.32 (br d, $J$ = 7.2 Hz, 1H), 5.20 (d, $J$ = 12.4 Hz, 1H), 5.15 (d, $J$ = 12.4 Hz, 1H), 4.34−4.24 (m, 1H), 2.52−2.39 (m, 2H), 2.21−2.11 (m, 1H), 2.02−1.92 (m, 1H), 1.40 (s, 9H); $^{13}C$ NMR (acetone-$d_6$, 125 MHz) $\delta$ 174.1, 172.9, 156.5, 137.1, 129.3 (2C), 128.9, 128.8 (2C), 79.4, 67.1, 54.1, 30.4, 28.5 (3C), 27.5; HRMS (ESI) $m/z$ 360.1417 $[M + Na]^+$ calcd for $C_{17}H_{23}NNaO_6$, 360.1418. $^1H$ NMR spectrum was in agreement with commercially available 1-benzyl *N*-Boc-L-glutamate and optical rotation was in agreement with that reported previously.[14]

*(R)-Benzyl 2-(tert-Butoxycarbonylamino)-5-hydroxypentanoate (**17**).* A stirred solution of **16** (1.53 g, 4.54 mmol) in 22 mL of dry THF under nitrogen atmosphere was cooled to −10 °C, to which DIEA (800 μL, 4.70 mmol) and ethyl chlorocarbonate (500 μL, 5.16 mmol) were added dropwise. The mixture was stirred for 30 min at the same temperature, and $NaBH_4$ (510 mg, 13.5 mmol) was added in one portion. Then the reaction mixture was allowed to warm slowly to rt, and $H_2O$ (10 mL) was added dropwise for 10 min. The mixture was stirred for additional 30 min and 10 mL of brine was added to it. The mixture was extracted with EtOAc (2 × 20 mL), and the combined organic layers were washed with 20 mL each of sat. aq $NaHCO_3$, sat. aq $NH_4Cl$, and brine, dried over anhydrous $Na_2SO_4$ and concentrated in vacuo. The residue was chromatographed ($SiO_2$, *n*-hexane/acetone = 5:1 to 1:5) to yield **17** (856 mg, 2.65 mmol, 58.3%) as a colorless oil: $[\alpha]^{20}_D$ +28.5 (*c* 2.8, MeOH); IR (neat) 3361 (br), 2977, 2936, 2876, 1737, 1709, 1518, 1455, 1391, 1366, 1252, 1213, 1165 $cm^{-1}$; $^1H$ NMR (acetone-$d_6$, 500 MHz) $\delta$ 7.44−7.30 (m, 5H), 6.29 (br d, $J$ = 6.8 Hz, 1H), 5.19 (d, $J$ = 12.4 Hz, 1H), 5.14 (d, $J$ = 12.4 Hz, 1H), 4.25−4.17 (m, 1H), 3.60−3.50 (m, 3H), 1.97−1.87 (m, 1H), 1.81−1.71 (m, 1H), 1.67−1.55 (m, 2H), 1.40 (s, 9H); $^{13}C$ NMR (acetone-$d_6$, 125 MHz) $\delta$ 173.4, 156.5, 137.3, 129.3 (2C), 128.9, 128.8 (2C), 79.2, 66.9, 61.8, 54.7, 30.0 (judged from HMQC spectra), 29.2, 28.6 (3C); HRMS (ESI) $m/z$ 346.1612 $[M + Na]^+$ calcd for $C_{17}H_{25}NNaO_5$, 346.1625.

*N-(Benzyloxy)-2-nitrobenzenesulfonamide (NsNHOBn).* To a stirred solution of *O*-benzyl hydroxylamine hydrochloride (2.40 g, 15.0 mmol) in dry pyridine (20 mL) at −5 °C under nitrogen atmosphere was added 2-nitrosulfonyl chloride (3.37 g, 15.2 mmol) in dry pyridine (10 mL) dropwise for 20 min. The mixture was stirred at the same temperature for 1 h, slowly warmed to rt, stirred for 4 h, mixed with $H_2O$, and concentrated in vacuo. The residue was dissolved in a little amount of $CHCl_3$ and recrystallized with MeOH. The crystals were washed with cold MeOH to give NsNHOBn (3.20 g, 10.4 mmol, 69.3%) as a colorless crystal: $^1H$ NMR ($CDCl_3$, 500 MHz) $\delta$ 8.27−8.23 (m, 1H), 8.12 (s, 1H), 7.89−7.85 (m, 1H), 7.81−7.75 (m, 2H), 7.40−7.34 (m, 5H), 5.08 (s, 2H); $^{13}C$ NMR ($CDCl_3$, 125 MHz) $\delta$ 148.6, 134.9, 134.9, 133.8, 133.0, 130.5, 129.6 (2C), 129.0, 128.7 (2C), 125.7, 79.9; HRMS (ESI) $m/z$ 331.0350 $[M + Na]^+$ calcd for $C_{13}H_{12}N_2NaO_5S$, 331.0359; mp 154 °C. $^1H$ NMR spectrum was in agreement with that reported previously.[15]

*(R)-Benzyl 11,11-Dimethyl-3-(2-nitrophenylsulfonyl)-9-oxo-1-phenyl-2,10-dioxa-3,8-diazadodecane-7-carboxylate (**18**).* To a mixture of alcohol **17** (825 mg, 2.55 mmol), NsNHOBn (827 mg, 2.68 mmol), and $PPh_3$ (700 mg, 2.67 mmol) in dry THF (25 mL) at 0 °C under nitrogen atmosphere was added 1.45 mL of 1.9 M DIAD in toluene dropwise for 15 min. After addition of DIAD, the mixture was allowed to warm to rt, stirred for 1 h, and quenched with sat. aq $NH_4Cl$ (15 mL). The aqueous layer was extracted with EtOAc (15 mL). The combined organic layers were washed with sat. aq $NH_4Cl$



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and brine, dried over anhydrous $Na_2SO_4$, and concentrated in vacuo. The residue was chromatographed on $SiO_2$ (*n*-hexane/EtOAc = 4:1 to 2:1 then $CHCl_3$/MeOH = 80:1) to yield **18** (1.44 g, 2.35 mmol, 92.0%) as a colorless oil: $[\alpha]^{20}_D$ +3.0 (*c* 0.13, MeOH); IR (neat) 2978, 1740, 1712, 1548, 1499, 1456, 1177 $cm^{-1}$; $^1H$ NMR (acetone-$d_6$, 500 MHz) $\delta$ 8.14−8.10 (m, 1H), 7.97−7.91 (m, 1H), 7.85−7.79 (m, 2H), 7.50−7.45 (m, 2H), 7.43−7.28 (m, 8H), 6.32 (d, *J* = 8.5 Hz, 1H), 5.19 (d, *J* = 12.3 Hz, 1H), 5.14 (d, *J* = 12.3 Hz, 1H), 5.14−5.05 (m, 2H), 4.29−4.20 (m, 1H), 3.16 (br s, 2H), 2.02−1.88 (m, 1H), 1.86−1.65 (m, 3H), 1.41 (s, 9H); $^{13}C$ NMR (acetone-$d_6$, 125 MHz) $\delta$ 172.9, 156.3, 150.5, 136.9, 136.5, 135.7, 133.1, 132.1, 130.6 (2C), 129.6, 129.3 (2C), 129.2 (2C), 128.8, 128.6 (2C), 125.9, 124.6, 80.8, 79.3, 67.0, 54.3, 53.7, 29.6, 28.5 (3C), 23.9; HRMS (ESI) *m*/*z* 652.1742 $[M + K]^+$ calcd for $C_{30}H_{35}N_3KO_9S$, 652.1726.

*(R)-Benzyl 11,11-Dimethyl-9-oxo-1-phenyl-2,10-dioxa-3,8-diazadodecane-7-carboxylate (**19**).* To a stirred mixture of compound **18** (1.37 g, 2.24 mmol) and $K_2CO_3$ (333 mg, 2.41 mmol) in dry DMF (11 mL) under nitrogen atmosphere was added PhSH (280 $\mu$L, 2.74 mmol). After being stirred for 50 min, the reaction was quenched with sat. aq $NH_4Cl$ (20 mL) and extracted with EtOAc (3 × 15 mL). Combined organic layers were washed with sat. aq $NH_4Cl$ and brine, dried over anhydrous $Na_2SO_4$, and concentrated in vacuo. The residue was chromatographed on $SiO_2$ (*n*-hexane/EtOAc = 10:3 to 4:3) to yield **19** (900 mg, 2.10 mmol, 93.8%) as a colorless oil: $[\alpha]^{20}_D$ +25.1 (*c* 1.3, MeOH); IR (neat) 3354 (br), 3032, 2976, 2931, 2868, 1713, 1498, 1455, 1365, 1249, 1161 $cm^{-1}$; $^1H$ NMR (acetone-$d_6$, 500 MHz) $\delta$ 7.42−7.22 (m, 10H), 6.29 (br d, *J* = 8.3 Hz, 1H), 6.10 (s, 1H), 5.19 (d, *J* = 12.4 Hz, 1H), 5.13 (d, *J* = 12.4 Hz, 1H), 4.64 (s, 2H), 4.25−4.16 (m, 1H), 2.89 (br t, *J* = 8.6 Hz, 2H), 1.94−1.85 (m, 1H), 1.81−1.70 (m, 1H), 1.68−1.55 (m, 2H), 1.40 (s, 9H); $^{13}C$ NMR (acetone-$d_6$, 125 MHz) $\delta$ 173.3, 156.5, 139.7, 137.3, 129.3 (2C), 129.1 (2C), 128.9 (2C), 128.9, 128.8 (2C), 128.2, 79.2, 76.6, 66.9, 54.7, 52.1, 29.5, 28.6 (3C), 24.5; HRMS (ESI) *m*/*z* 451.2204 $[M + Na]^+$ calcd for $C_{24}H_{32}N_2NaO_5$, 451.2203.

*(R)-Benzyl 3-Formyl-11,11-dimethyl-9-oxo-1-phenyl-2,10-dioxa-3,8-diazadodecane-7-carboxylate (**20**).* A mixture of acetic anhydride (400 $\mu$L, 4.26 mmol) and formic acid (320 $\mu$L, 8.48 mmol) was stirred for 1 h, and this mixture was poured into a solution of compound **19** (886 mg, 2.07 mmol) in dry DCM (10 mL). After being stirred for 1.5 h, the solution was washed with sat. aq $NaHCO_3$ (20 mL), dried over anhydrous $Na_2SO_4$, and concentrated in vacuo to give **20** (939 mg, 2.06 mmol, 99.5%) as a colorless oil: $[\alpha]^{20}_D$ +14.9 (*c* 1.2, MeOH); IR (neat) 3336 (br), 2974, 2938, 2876, 1744, 1710, 1679, 1508, 1456, 1365, 1251, 1212, 1162 $cm^{-1}$; $^1H$ NMR (acetone-$d_6$, 500 MHz) $\delta$ 8.22 (br s, 1H), 7.49−7.44 (m, 2H), 7.42−7.29 (m, 8H), 6.33 (br d, *J* = 8.2 Hz, 1H), 5.18 (d, *J* = 12.3 Hz, 1H), 5.13 (d, *J* = 12.3 Hz, 1H), 4.94 (s, 2H), 4.27−4.21 (m, 1H), 3.72−3.48 (m, 2H), 1.91−1.81 (m, 1H), 1.80−1.68 (m, 3H), 1.39 (s, 9H); $^{13}C$ NMR (acetone-$d_6$, 125 MHz) $\delta$ 173.0, 163.4, 156.4, 137.1, 130.4 (2C), 129.5, 129.3, 129.2, 128.8, 128.7 (9C from 129.5 to 128.7), 79.3, 77.9, 66.9, 54.3, 43.9, 29.5, 28.5 (3C), 24.1; HRMS (ESI) *m*/*z* 479.2166 $[M + Na]^+$ calcd for $C_{25}H_{32}N_2NaO_6$, 479.2153.

**Synthesis of Chlorocatechelin A (1).** *Hexabenzyl-chlorocatechelin A (**21**).* To a solution of compound **20** (89.6 mg, 0.196 mmol) in dry DCM (400 $\mu$L) was added TFA (400 $\mu$L). After being stirred for 1 h, the solution was concentrated in vacuo. This material was mixed with compound **4** (125.4 mg, 0.143 mmol) in dry DMF (200 $\mu$L), to which DIEA (100 $\mu$L, 0.588 mmol), HATU (60.6 mg, 0.159 mmol), and HOAt (20.6 mg, 0.151 mmol) were added at 0 °C under nitrogen atmosphere. After being stirred for 30 min at the same temperature, the mixture was allowed to warm slowly to rt, stirred for 2.5 h, and quenched with sat. aq $NH_4Cl$. The aqueous solution was extracted with EtOAc twice, and combined organic layers were washed with sat. aq $NH_4Cl$ and brine, dried over anhydrous $Na_2SO_4$, and concentrated in vacuo. The residue was chromatographed on $SiO_2$ ($CHCl_3$/acetone/MeOH = 50:10:1 to 20/10/1) to yield a mixture of **21** and an unseparable compound (153.9 mg). This mixture was used in the next reaction without further purification. Analytical samples were obtained after purification on reversed-phase HPLC (Cosmosil 5C18-AR-II, 250 × 20 mm, $H_2O$/MeCN containing 0.1% TFA (25:75)) as a colorless amorphous solid: $[\alpha]^{20}_D$ −3.3 (*c* 0.45, MeOH); IR (neat) 3299 (br), 3065, 3033, 2930, 2876, 1675, 1579, 1518, 1455, 1429, 1365, 1289, 1202, 1081 $cm^{-1}$; $^1H$ NMR (acetone-$d_6$, 500 MHz)[16] $\delta$ 8.62−8.35 (m, 1H), 8.19 (br s, 1H), 7.97 (br s, 1H), 7.74 (d, *J* = 8.6 Hz, 1H), 7.55−7.24 (m, 32H), 7.15 (d, *J* = 8.4 Hz, 1H), 5.40−5.10 (m, 6H), 5.06 (s, 2H), 5.02 (s, 2H), 4.89 (s, 2H), 4.84−4.68 (m, 1H), 4.64−4.54 (m, 1H), 3.70−3.40 (m, 2H), 3.36−3.12 (m, 2H), 1.98−1.80 (m, 2H), 1.8−1.68 (m, 3H), 1.68−1.50 (m, 3H); $^{13}C$ NMR (acetone-$d_6$, 125 MHz)[16] $\delta$ 172.4, 164.6, 163.5, 162.4, 152.7, 149.9, 149.5, 138.9, 138.1, 137.5, 137.0, 136.9, 132.9, 130.6−128.5, 128.1, 127.4, 126.2, 125.2, 78.0, 77.4, 76.6, 76.1, 75.9, 67.3, 53.5, 53.1, 43.9, 41.2, 31.3, 29.4 (judged from the HMQC spectrum), 25.8, 24.2; HRMS (ESI) *m*/*z* 1213.4253 $[M + H]^+$ calcd for $C_{68}H_{66}Cl_2N_6O_{11}$, 1213.4239.

*Pentabenzyl-chlorocatechelin A (**22**).* A mixture material described above (127.2 mg) was dissolved in THF (2.5 mL) and hydrolyzed with 1.2 mL of 1 M LiOH for 20 min. After being quenched with 1.25 mL of 1 N aq HCl and brine, the mixture was extracted with EtOAc (2 × 15 mL). The combined organic layers were washed with $H_2O$ and brine, dried over anhydrous $Na_2SO_4$, and concentrated in vacuo. The residue was chromatographed on $SiO_2$ ($CHCl_3$/acetone/MeOH = 20:10:1, 4:0:1) followed by purification on RP-HPLC (Cosmosil 5C18-AR-II, 250 × 20 mm, $H_2O$/MeCN containing 0.1% TFA (30:70)) to yield **22** (86.9 mg, 0.0773 mmol, 65.4% in two steps (based on compound **4**)) as a colorless amorphous solid: $[\alpha]^{20}_D$ −2.8 (*c* 1.7, MeOH); IR (neat) 3299 (br), 3064, 3033, 2946, 1670, 1580, 1456, 1429, 1366, 1290, 1202, 1135 $cm^{-1}$; $^1H$ NMR ($CD_3OD$, 500 MHz) $\delta$ 8.07 and 7.96 (s, 1H), 7.60 (d, *J* = 8.4 Hz, 1H), 7.55−7.49 (m, 2H), 7.47 (d, *J* = 8.6 Hz, 1H), 7.45−7.22 (m, 24H), 7.17 (d, *J* = 7.3 Hz, 2H), 5.23 (d, *J* = 10.8 Hz, 1H), 5.20 (s, 2H), 5.14 (s, 2H), 5.13 (d, *J* = 10.9 Hz, 1H), 4.99 (s, 2H), 4.84 (overlapped with HDO), 4.71−4.60 (m, 1H), 4.55−4.45 (m, 1H), 3.70−3.38 (m, 2H), 3.33−3.20 (m, 2H), 2.02−1.84 (m, 2H), 1.82−1.63 (m, 6H); $^{13}C$ NMR ($CD_3OD$, 125 MHz) $\delta$ 174.9, 173.3, 167.2, 166.5, 164.8, 160.4, 154.5, 152.9, 152.5, 150.3, 149.9, 137.7, 137.7, 137.2, 136.3, 136.2, 133.9, 131.0−129.3, 128.2, 127.4, 127.4, 127.0, 127.0, 126.7, 78.8, 78.4, 77.8, 77.1, 76.8, 76.6, 54.2, 53.3, 44.3, 42.2, 30.9, 29.7, 29.5, 25.1, 24.5; HRMS (ESI) *m*/*z* 1123.3786 $[M + H]^+$ calcd for $C_{61}H_{61}Cl_2N_6O_{11}$, 1123.3770.

*Chlorocatechelin A (**1**).* To a solution of compound **22** (44.2 mg, 0.0393 mmol) in THF (2 mL) was added 10% Pd/C (22.8 mg), and the mixture was stirred under hydrogen. After being stirred for 2 h, the catalyst was removed with Celite. The filtrate was concentrated in vacuo and purified on RP-HPLC (Senshu Pak PEGASIL ODS SP100, 250 × 20 mm, $H_2O$/MeCN (80:20 to 0:100)) to give **1** (13.5 mg, 0.0200 mmol, 51.0%) as a brown amorphous solid: $[\alpha]^{20}_D$ +3.9 (*c* 0.24, MeOH) ; $^1H$ NMR (DMSO-$d_6$, 500 MHz) $\delta$ 9.30 (br, 1H), 9.02 (br, 1H), 8.37 (br d, *J* = 5.2 Hz, 1H) 8.25 and 7.90 (s, 1H (combined)), 7.48 (d, *J* = 8.4 Hz, 1H), 7.24 (d, *J* = 8.1 Hz, 1H), 6.87 (d, *J* = 8.4 Hz, 1H), 6.60 (d, *J* = 8.1 Hz, 1H), 4.60−4.50 (m, 1H), 4.26−4.15 (m, 1H), 3.51−3.35 (m, 2H), 3.35−3.18 (m, 2H), 1.94−1.50 (m, 8H); $^{13}C$ NMR (DMSO-$d_6$, 125 MHz) $\delta$ 173.4, 173.0, 171.2, 169.0, 161.8 (157.1), 158.4, 153.6, 151.1, 142.9, 142.6, 123.6, 121.8, 119.5, 118.4, 118.3, 116.8, 116.1, 113.6, 52.7, 51.8 (51.7), 48.7 (45.4), 40.4, 29.0, 28.1 (27.7), 25.0, 23.4 (22.9); HRMS (ESI) *m*/*z* 673.1444 $[M + H]^+$ calcd for $C_{26}H_{31}Cl_2N_6O_{11}$, 673.1422.

**Antimicrobial Assay.** Antimicrobial activities of **1**, **2**, VCM, and DFB were tested with an agar dilution streak method (2-fold dilution). Microbes listed in Table 1 were incubated in culture medium 1 (1/3 brain-heart infusion broth and 2% NaCl) with test compounds at 27 °C for 18 h. Microbes in Table 2 were incubated in culture medium 2 (5% polypeptone) with test compounds at 37 °C for 18 h.

## ■ ASSOCIATED CONTENT

### Supporting Information

$^1H$ and $^{13}C$ NMR spectra for synthesized products. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.5b00532.



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### Notes

The authors declare no competing financial interests.

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