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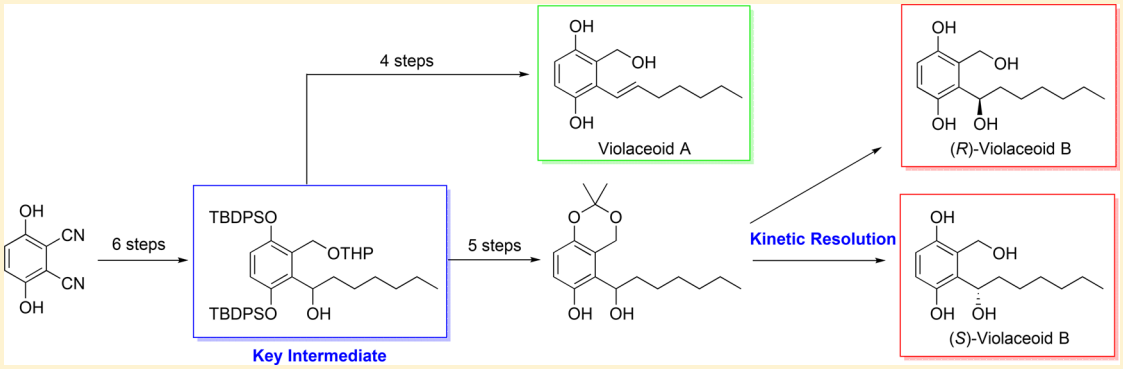
Total Synthesis of Violaceoid A and (−)- and (+)-Violaceoid B

Takatsugu Murata,[†] Teppei Kuboki,[†] Ryo Ishikawa,[†] Takahiro Saito,[†] Shotaro Taguchi,[†] Kazuma Takeuchi,[†] Emiko Hatano,[†] Motoyuki Shimonaka,[‡] and Isamu Shiina*[,†]

[†]Department of Applied Chemistry, Faculty of Science, Tokyo University of Science, 1-3 Kagurazaka, Shinjuku-ku, Tokyo 162-8601, Japan

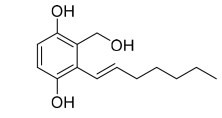
[‡]Department of Chemistry, Faculty of Science, Tokyo University of Science, 1-3 Kagurazaka, Shinjuku-ku, Tokyo 162-8601, Japan

Supporting Information

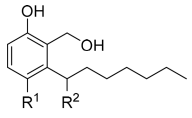


ABSTRACT: The first total synthesis of violaceoid A, a cytotoxic agent, and the asymmetric total synthesis of (−)- and (+)-violaceoid B are reported. The precursor was accessed by desymmetrization of a substituted quinol moiety, and the racemic secondary alcohol was kinetically resolved using a chiral nucleophilic catalyst. The asymmetric synthesis of (−)- and (+)-violaceoid B elucidated the absolute configuration of the naturally occurring violaceoid B. Synthetic violaceoid A inhibited the growth of human breast cancer cell lines MCF-7 and Hs 578T at concentrations of less than 100 μM, while (*S*)- and (*R*)-violaceoid B were inactive.

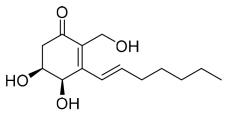
In 2014, Sugawara and co-workers reported a series of unique alkylated hydroquinones, violaceoids A−F (compounds **1**−**6**), which were isolated from a culture broth of *Aspergillus violaceofuscus* Gasperini coexisting with moss.[1] Violaceoids B and D−F are chiral compounds, and the absolute configurations of violaceoids B, D, and E have not yet been determined. Sugawara's group also reported that violaceoids exhibit cytotoxicity. Among them, violaceoid A (**1**) inhibited the growth of several human cancer cell lines. In addition, neither the total synthesis nor the synthetic approach for violaceoids A−E has ever been reported to the best of our knowledge.[2] Considering this, we initiated a program to synthesize **1** and **2**, and the details are presented in this paper.



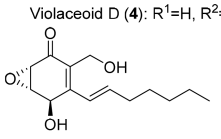
Violaceoid A (**1**)



Violaceoid B (**2**): R^1=OH, R^2=OH
Violaceoid C (**3**): R^1=OH, R^2=H
Violaceoid D (**4**): R^1=H, R^2=OH



Violaceoid E (**5**)



Violaceoid F (**6**)

RESULTS AND DISCUSSION

The retrosynthetic analysis of **1** and **2** is shown in Scheme 1.

Optically active (*S*)- or (*R*)-violaceoid B (**2**) can be obtained by the deprotection of the acetonide group from (*S*)- or (*R*)-**7**, respectively. Diols (*S*)- and (*R*)-**7** can be separated by using a kinetic resolution method of a racemic compound (*rac*-**7**) that our group has already reported.[3] The substrate for kinetic resolution can be obtained from the racemic secondary alcohol **8**, which can be prepared from 3,6-dihydroxyphthalonitrile (**11**) via direct protection of **11**.

Following this strategy, we first tried to protect two hydroxy groups of 3,6-dihydroxyphthalonitrile as *tert*-butyldiphenylsilyl (TBDPS) ether depicted in Scheme 2. However, we could not obtain the desired phthalonitrile **10**. Then we conducted the protection transforming it into methoxymethyl (MOM) ether groups, but we could get the desired compound **12** in low yield. On the basis of the above results, we decided to change the synthetic plan for providing violaceoids A and B as shown in Scheme 3.

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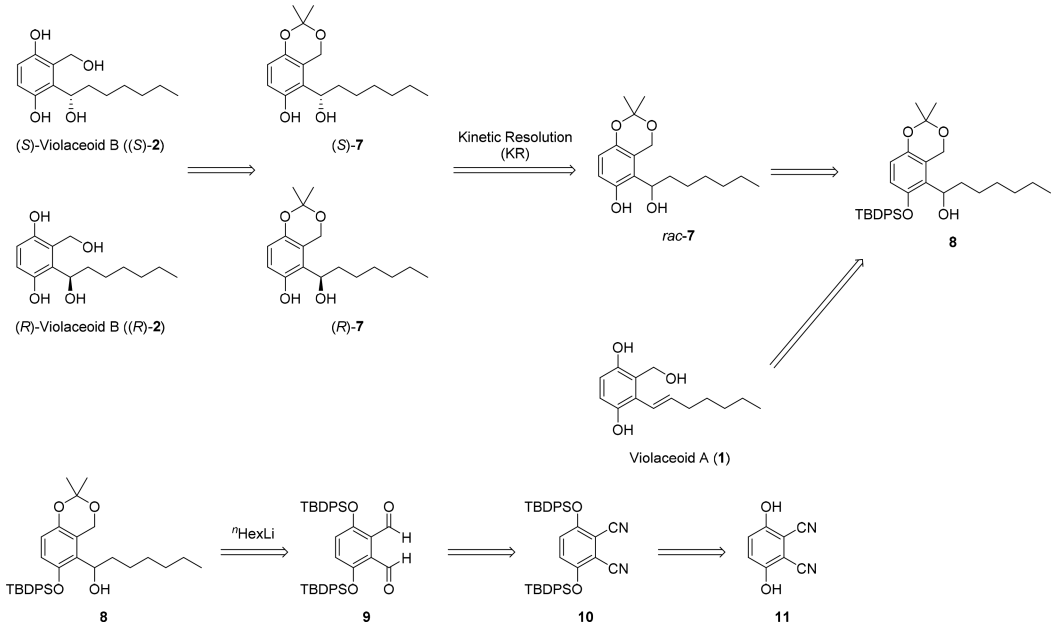
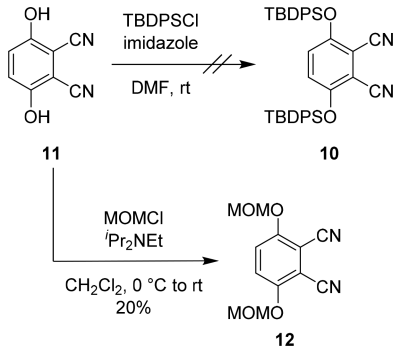
Scheme 1. Retrosynthetic Analysis of 1 and 2



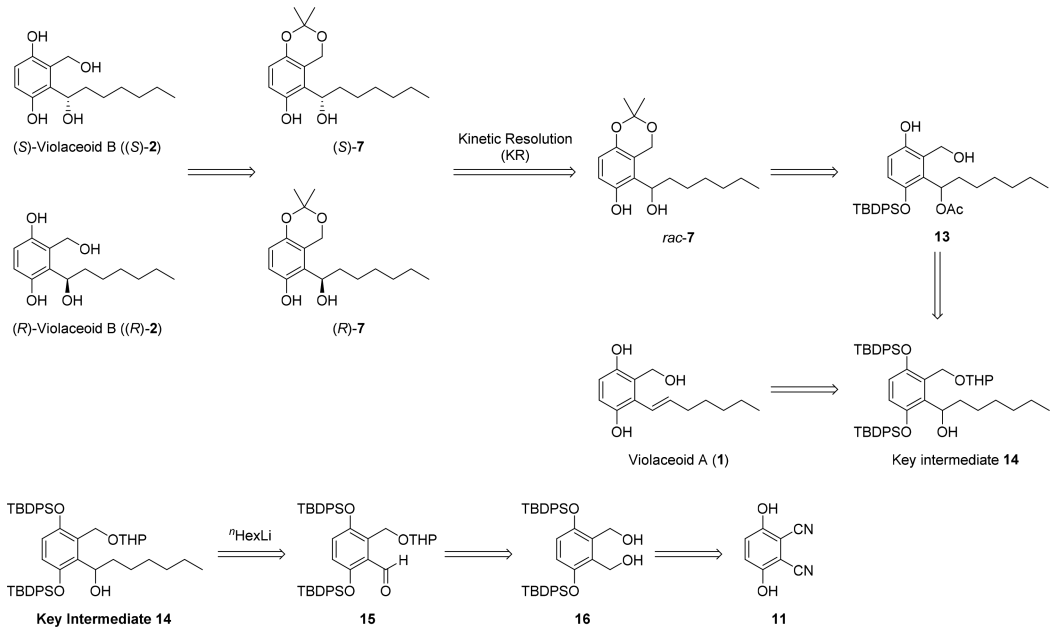
Scheme 2. Protection of 3,6-Dihydroxyphtalonitrile (11)



In the revised synthetic plan, *rac*-**7**, the substrate for kinetic resolution, can be obtained from the racemic secondary alcohol **14** by several functional transformations. Violaceoid A (**1**) can also be derived from **14**. The key intermediate **14** can be prepared from **11** via reduction of a derivative of **11**.

The preparation of **14** was carried out as depicted in Scheme 4. The hydrolysis of **11** followed by esterification gave the dimethyl ester **17**.[4] The two hydroxy groups of **17** were protected with TBDPS groups to afford **18**. The symmetric diol **16** was obtained by the reduction of diester **18**. Monotetrahydropyranylation gave the unilateral protected alcohol **19**. After the oxidation of alcohol **19** to aldehyde **15** followed by alkylation with an alkyl lithium reagent, we obtained the key intermediate **14**.

Scheme 3. Revised Retrosynthetic Analysis of 1 and 2



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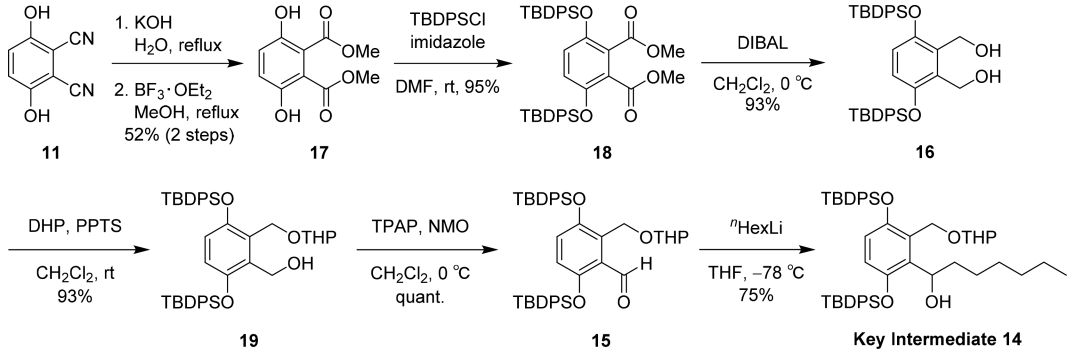
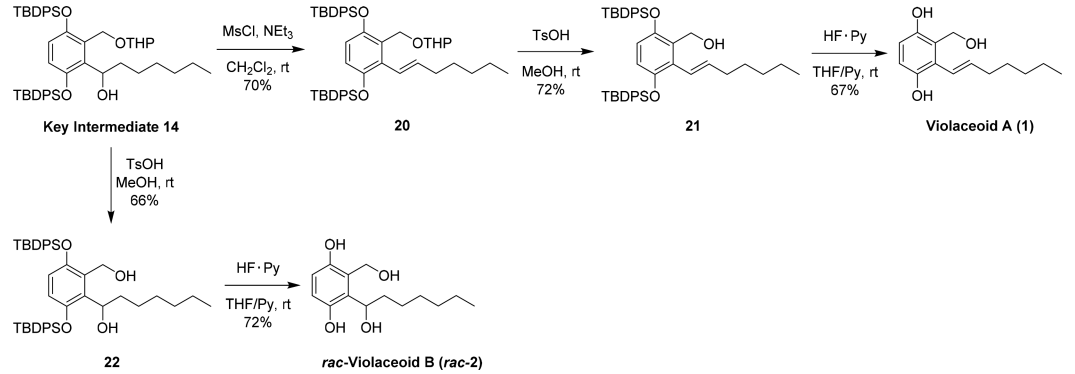
Scheme 4. Synthesis of Key Intermediate 14



Scheme 5. Synthesis of 1 and *rac*-2



We next attempted to synthesize **1** and *rac*-**2** (Scheme 5).

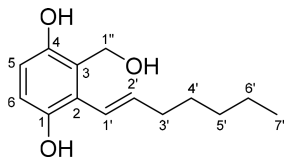
The double bond in **20** was generated by mesylation of **14**. Then, the THP group was removed, and we obtained the alcohol **21**. Finally, by deprotection of the two TBDPS groups, we accomplished the total synthesis of violaceoid A (**1**). In addition, by sequential deprotection of the THP and TBDPS groups of **14**, we achieved the synthesis of *rac*-violaceoid B (*rac*-**2**). We compared the ^{1}H and ^{13}C NMR data of the synthetic **1** and **2** with those of naturally occurring violaceoids A and B reported in the literature to determine the true structure. The results are shown in Tables 1 and 2. As a result, the ^{1}H and ^{13}C NMR data of synthetic **1** and **2** were shown to be in accordance with those reported for the natural compounds.

Subsequently, we attempted to determine the absolute configuration of naturally occurring violaceoid B (**2**), as depicted in Scheme 6.

We obtained the alcohol **24** by acetylation of **14** followed by deprotection of the THP group. Next, deprotection of the TBDPS group was conducted to afford the 1,3-diol **13**. The acetyl and TBDPS groups were reductively removed together to yield *rac*-**7**, which is the substrate for kinetic resolution.[3] The kinetic resolution was conducted to afford the enantiorich ester (*R*)-**26**[5] and enantiorich alcohol (*S*)-**7**. Further, the same reaction was repeated five times to obtain the enantiorich alcohol in 95% ee. Finally, by deprotection of the acetonide group, we accomplished the asymmetric total synthesis of (*S*)-violaceoid B ((*S*)-**2**). However, the optical rotation of (*S*)-**2** was not consistent with the natural product, which motivated us to synthesize the enantiomer, (*R*)-violaceoid B ((*R*)-**2**) (Scheme 7).

As for enantiopure (*S*)-**7**, we conducted the kinetic resolution procedure. Using the recovered enantiorich alcohol, the same reaction was then repeated five times to obtain the enantiorich alcohol in 93% ee. Finally, deprotection of the

Table 1. Comparison of the ^{1}H and ^{13}C NMR Data of Naturally Occurring Violaceoid A with Those of Synthetic 1 in CD_3OD



	natural violaceoid A[a]			synthetic violaceoid A	
position	δ_C	δ_H (J in Hz)	position	δ_C[b]	δ_H (J in Hz)[c]
1	148.8		1	148.8	
2	127.6		2	127.6	
3	125.9		3	125.9	
4	150.6		4	150.6	
5	115.0	6.57, d (8.7)	5	114.9	6.54, d (8.7)
6	116.3	6.60, d (8.7)	6	116.2	6.59, d (8.7)
1′	124.6	6.45, dt (16.1, 6.9)	1′	124.6	6.50−6.42, m
2′	137.9	6.06, dt (16.1, 6.9)	2′	137.8	6.08, dt (15.6, 6.9)
3′	34.9	2.24, tdd (6.9, 6.9, 1.5)	3′	34.9	2.32−2.19, m
4′	30.3	1.51, m	4′	30.4	1.61−1.43, m
5′	32.7	1.37, m	5′	32.7	1.43−1.30, m
6′	23.6	1.37, m	6′	23.7	1.43−1.30, m
7′	14.4	0.93, t (7.1)	7′	14.5	0.94, t (7.2)
1″	58.5	4.72, s	1″	58.4	4.71, s

[a]300 MHz for ^{1}H NMR and 75 MHz for ^{13}C NMR. See ref 1. [b]125 MHz using JNM-LA500. [c]300 MHz using JNM-AL300.

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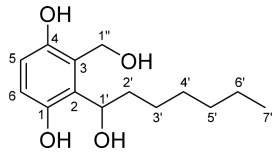
Table 2. Comparison of the ^{1}H and ^{13}C NMR Data of Naturally Occurring Violaceoid B with Those of Synthetic 2 in CD_3OD



	natural violaceoid B[a]			synthetic violaceoid B	
position	δ_C	δ_H (J in Hz)	position	δ_C[b]	δ_H (J in Hz)[c]
1	150.1		1	150.1	
2	130.4		2	130.5	
3	125.1		3	125.0	
4	150.1		4	150.0	
5	115.8	6.57, d (8.7)	5	115.6	6.63, d (8.4)
6	117.5	6.60, d (8.7)	6	117.5	6.60, d (8.4)
1′	72.0	5.15, dd (9.0, 4.6)	1′	71.9	5.19, dd (9.2, 4.4)
2′	38.2	1.89, m, 1.71, m	2′	38.2	2.00−1.83, 1.80−1.67, m
3′	27.2	1.55, m	3′	27.2	1.67−1.43, m
4′	30.3	1.33, m	4′	30.4	1.43−1.25, m
5′	33.1	1.33, m	5′	33.1	1.43−1.25, m
6′	23.7	1.33, m	6′	23.7	1.43−1.25, m
7	14.4	0.89, t (6.8)	7′	14.5	0.93, t (6.8)
1″	56.6	4.71, d (11.7), 4.68, d (11.7)	1″	56.5	4.75, d (11.6), 4.71, d (11.6)

[a]300 MHz for ^{1}H NMR and 75 MHz for ^{13}C NMR. See ref 1. [b]125 MHz using JNM-LA500. [c]400 MHz using AVANCE 400M.

acetonide group was conducted, and we accomplished the asymmetric total synthesis of (*R*)-violaceoid B ((*R*)-**2**). The optical rotation of synthetic (*R*)-**2** was consistent with that reported for naturally occurring violaceoid B.

To evaluate the antiproliferative effect of violaceoids on human breast cancer cells, MCF-7 cells or Hs 578T cells were incubated with synthetic violaceoid A and (*S*)- and (*R*)-violaceoid B, and cell numbers were estimated using WST-8 reagent. Violaceoid A (**1**) inhibited the growth of MCF-7 cells and Hs 578T cells at concentrations of 10−100 μM in a dose-dependent manner. The GI_{50} values of **1** in MCF-7 cells and Hs 578T cells were 61.5 ± 18.0 and 59.7 ± 10.0 μM, respectively. On the other hand, (*S*)-violaceoid B ((*S*)-**2**) and (*R*)-violaceoid B ((*R*)-**2**) did not inhibit the growth of these cells within the same range. The GI_{50} values of (*S*)-**2** and (*R*)-**2** in these cells were therefore estimated as >100 μM.

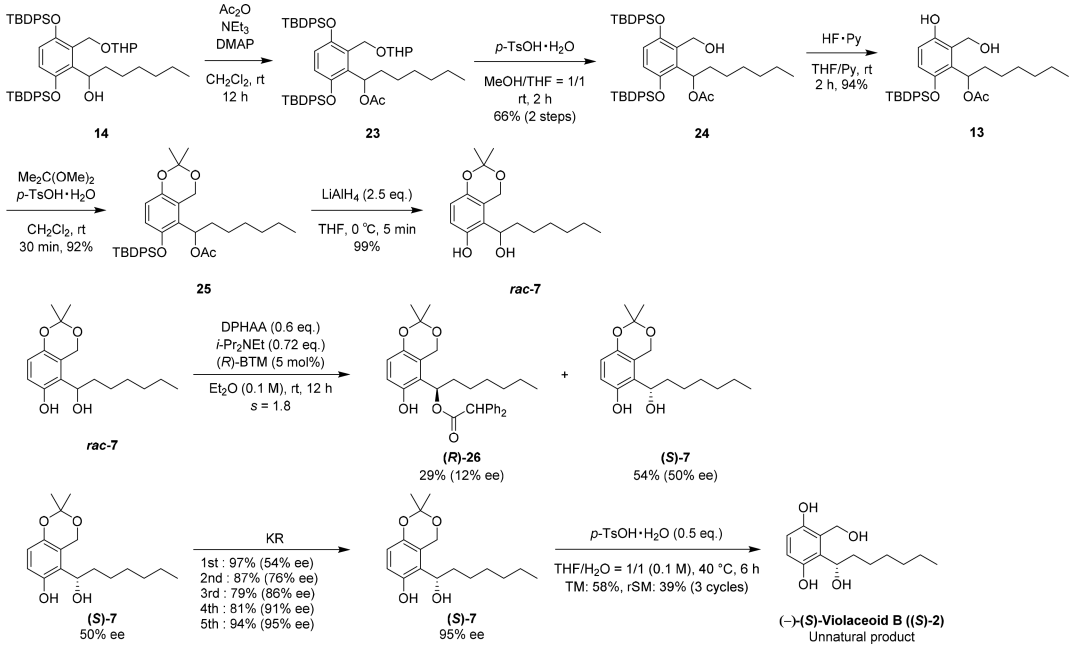
In conclusion, we have accomplished the first total synthesis of violaceoids A and B and successfully elucidated the absolute configuration of the naturally occurring violaceoid B. The cytotoxicity of the synthetic violaceoid A and (*S*)- or (*R*)-violaceoid B against human cancer cells was assessed using MCF-7 cells or Hs 578T cells, and it was shown that violaceoid A inhibited the growth of both breast cancer cell lines at concentrations of less than 100 μM (with GI_{50} values of 61.5 ± 18.0 μM for MCF-7 and 59.7 ± 10.0 μM for Hs 578T).

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were determined using a Jasco P-1020 polarimeter. Infrared (IR) spectra were obtained using a Jasco FT/IR-4600 Fourier transform infrared spectrometer. ^{1}H and ^{13}C NMR spectra were recorded with chloroform (in $CDCl_3$) or methanol (in CD_3OD) on the following instrument: JEOL JNM-AL300 (^{1}H at 300 MHz and ^{13}C at 75 MHz), JEOL JNM-LA500 (^{1}H at 500 MHz and ^{13}C at 125 MHz), Bruker Biospin AVANCE 400 M (^{1}H at 400 MHz and ^{13}C at 100 MHz). Mass spectra were determined by a Bruker Daltonics micrOTOF focus (ESI-TOF) mass spectrometer. Thin-layer chromatography was performed on Wakogel B-5F. HPLC was performed with a Hitachi LaChrom Elite system composed of the organizer, L-2400 UV detector, and L-2130 pump.

All reactions were carried out under an argon atmosphere in dried glassware unless otherwise noted. CH_2Cl_2 was distilled from diphosphorus pentoxide and then calcium hydride and dried over MS 4 Å. All reagents were purchased from Tokyo Kasei Kogyo Co., Ltd., Kanto Chemical Co., Inc., or Aldrich Chemical Co., Inc., and used without further purification unless otherwise noted. Carbon

Scheme 6. Synthesis of (*S*)-Violaceoid B ((*S*)-2)



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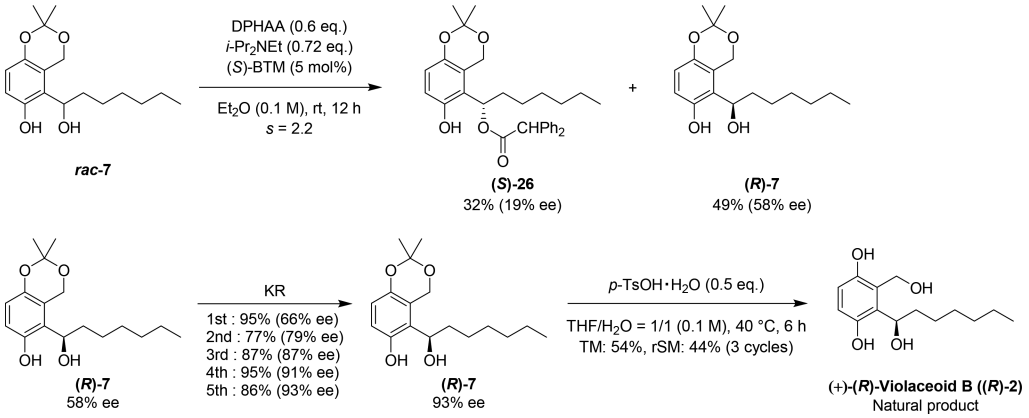
Scheme 7. Synthesis of (*R*)-Violaceoid B ((*R*)-2)



atoms of all compounds are numbered according to IUPAC nomenclature.

3,6-Bis(methoxymethoxy)phthalonitrile (12). To a solution of 3,6-dihydroxyphthalonitrile (**11**) (400 mg, 2.56 mmol) in CH_2Cl_2 (25.6 mL) were added diisopropylethylamine (1.76 mL, 10.24 mmol) and methyloxymethyl chloride (0.77 mL, 10.24 mmol) at 0 °C. The reaction mixture was stirred for 1 h and warmed up to room temperature (rt) and stirred for 12 h. The solution was diluted with water and extracted with CH_2Cl_2. The organic layer was washed with H_2O and brine, dried over Na_2SO_4, filtrated, and concentrated. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc = 4:1). Compound **12** (127 mg, 20%) was obtained as a white solid.

Dimethyl 3,6-Dihydroxyphthalate (17). To a solution of KOH (32.0 g, 570 mmol) in water (32.0 mL) was added 3,6-dihydroxyphthalonitrile (**11**) (5.00 g, 31.2 mmol) at rt. The reaction mixture was refluxed for 1 h. Aqueous 20% H_2SO_4 (100 mL) was slowly poured into the reaction mixture and extracted with Et_2O and $CHCl_3$ sequentially. The organic layer was dried over Na_2SO_4, filtrated, and concentrated in vacuo, giving 3,6-dihydroxyphthalic acid (6.20 g) as the crude product including starting material **11**. The mixture was used for the next reaction without further purification.

To a solution of the crude 3,6-dihydroxyphthalic acid (6.20 g) in MeOH (240 mL) was added boron trifluoride diethyl etherate (12.8 mL, 100 mmol) at room temperature, and reaction mixture was refluxed for 15 h. The solution was cooled to rt, concentrated in vacuo, quenched with saturated aqueous $NaHCO_3$, and extracted with EtOAc. The organic layer was dried over Na_2SO_4, filtrated, and concentrated in vacuo to yield dimethyl 3,6-dihydroxyphthalate (**17**) (3.69 g, 52% in two steps) as a white solid.

Dimethyl 3,6-Bis((*tert*-butyldiphenylsilyl)oxy)phthalate (18). 3,6-Dihydroxyphthalate (**17**) (500 mg, 2.21 mmol) was dissolved in CH_2Cl_2 (22 mL) and cooled to 0 °C. Imidazole (1.20 g, 17.68 mmol) and TBDPSCl (2.27 mL, 8.84 mmol) were added to the solution, and the reaction mixture was stirred for 2 h at rt. The mixture was quenched by saturated aqueous $NaHCO_3$ at 0 °C, extracted with EtOAc, washed with brine, and concentrated. The residue was purified by silica gel column chromatography (gradient, *n*-hexane/EtOAc = 20:1−5:1). Compound **18** (1.61 g, 95%) was obtained as a white solid.

(3,6-Bis((*tert*-butyldiphenylsilyl)oxy)-1,2-phenylene)-dimethanol (16). To a solution of **18** in CH_2Cl_2 was added diisobutylaluminum hydride (42.7 mL, 42.7 mmol) at −78 °C, and the reaction mixture was stirred at 0 °C for 2 h. The reaction mixture was quenched by MeOH and saturated aqueous Rochell's salt, extracted with CH_2Cl_2, washed with brine, and concentrated. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc = 10:1). Compound **16** (1.60 g, 93%) was obtained as a colorless solid.

(3,6-Bis((*tert*-butyldiphenylsilyl)oxy)-2-(((tetrahydro-2*H*-pyran-2′-yl)oxy)methyl)phenyl)methanol (19). To a solution of **16** (1.16 g, 1.79 mmol) in CH_2Cl_2 (44.8 mL, 0.04 M) were added pyridinium *p*-toluenesulfonate (90.4 mg, 0.36 mmol) and 3,4-dihydro-2*H*-pyran (0.23 mL, 2.69 mmol), and the reaction mixture was stirred for 4 h at rt. The reaction mixture was quenched by saturated aqueous $NaHCO_3$, extracted with CH_2Cl_2, and concentrated. The residue was purified by silica gel column chromatography (gradient, *n*-hexane/EtOAc = 20:1−1:1). Compound **19** (1.22 g, 93%) was obtained as a white solid.

3,6-Bis((*tert*-butyldiphenylsilyl)oxy)-2′-(((tetrahydro-2*H*-pyran-2-yl)oxy)methyl)benzaldehyde (15). To a solution of **19** (198 mg, 0.271 mmol) in CH_2Cl_2 (5.42 mL, 0.05 M) were added tetrapropylammonium perruthenate (28.6 mg, 0.0813 mmol) and 4-methylmorpholine *N*-oxide (95.5 mg, 0.813 mmol), and the reaction mixture was stirred for 2 h at 0 °C. The reaction mixture was filtrated through a short pad of silica gel with EtOAc, and the filtrate was concentrated. Compound **15** (197 mg, quant.) was obtained as a white solid.

1-(3′,6′-Bis((*tert*-butyldiphenylsilyl)oxy)-2′-(((tetrahydro-2*H*-pyran-2″-yl)oxy)methyl)phenyl)heptan-1-ol (14). To a solution of **15** (457 mg, 0.628 mmol) in THF (12.6 mL, 0.05 M) was added a 1.15 M hexyllithium solution in *n*-hexane at −78 °C. The reaction mixture was stirred for 5 min, quenched with saturated aqueous $NaHCO_3$, extracted with EtOAc, and concentrated. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc = 5:1). Compound **15** (382 mg, 75%) was obtained as a white solid.

(*E*)-((2-(Hept-1′-en-1′-yl)-3-(((tetrahydro-2*H*-pyran-2″-yl)-oxy)methyl)-1,4-phenylene)bis(oxy))bis(*tert*-butyl-diphenylsilane) (20). To a solution of **14** (180 mg, 0.220 mmol) in CH_2Cl_2 (4.35 mL, 0.05 M) were added methanesulfonyl chloride (0.047 mL, 0.660 mmol) and triethylamine (0.186 mL, 1.321 mmol) at 0 °C. The reaction mixture was stirred for 3 h at rt, quenched with saturated aqueous $NaHCO_3$, extracted with CH_2Cl_2, and concentrated. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc = 5:1). Compound **20** (122 mg, 70%) was obtained as a white solid.

(*E*)-(3,6-Bis((*tert*-butyldiphenylsilyl)oxy)-2-(hept-1′-en-1′-yl)phenyl)methanol (21). To a solution of **20** (84.0 mg, 0.106 mmol) in MeOH (4.24 mL, 0.025 M) was added *p*-toluenesulfonic acid monohydrate (30.0 mg, 0.158 mmol) at 0 °C. The reaction mixture was stirred for 2 h at rt, diluted with water, extracted with chloroform, and concentrated. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc = 5:1). Compound **21** (53.8 mg, 72%) was obtained as a colorless oil.

(*E*)-2-(Hept-1′-en-1′-yl)-3-(hydroxymethyl)benzene-1,4-diol (Violaceoid A (1)). To a solution of **21** (18.5 mg, 0.026 mmol) in THF/pyridine (1.7 mL, v/v = 1/1, 0.015 M) was added hydrogen fluoride pyridine complex (0.60 mL) at 0 °C. The reaction mixture was stirred for 2 h, quenched with saturated aqueous $NaHCO_3$, extracted with EtOAc, and washed with saturated aqueous copper sulfate, water, saturated aqueous $NaHCO_3$, and brine. The organic layer was dried over Na_2SO_4 and concentrated. The residue was

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purified by silica gel chromatography (*n*-hexane/EtOAc = 2:1). Violaceoid A (**1**) (4.1 mg, 67%) was obtained as a white solid.

1-(3′,6′-Bis((*tert*-butyldiphenylsilyl)oxy)-2′-(hydroxymethyl)phenyl)heptan-1-ol (22). To a solution of **14** (200 mg, 0.246 mmol) in MeOH (5.00 mL, 0.05 M) was added *p*-toluenesulfonic acid monohydrate (56.0 mg, 0.295 mmol) at 0 °C. The reaction mixture was stirred for 2 h at rt, diluted with water, extracted with chloroform, and concentrated. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc = 5:1). Compound **22** (119 mg, 66%) was obtained as a white solid.

2-(1′-Hydroxyheptyl)-3-(hydroxymethyl)benzene-1,4-diol (*rac*-Violaceoid B (2)). To a solution of **22** (77 mg, 0.105 mmol) in THF/pyridine (5.25 mL, v/v = 1:1, 0.02 M) was added hydrogen fluoride pyridine complex (2.00 mL) at 0 °C. The reaction mixture was stirred for 2 h, quenched with saturated aqueous $NaHCO_3$, extracted with EtOAc, and washed with saturated aqueous copper sulfate, water, saturated aqueous $NaHCO_3$, and brine. The organic layer was dried over Na_2SO_4 and concentrated. The residue was purified by silica gel chromatography (*n*-hexane/EtOAc = 2:1). *rac*-Violaceoid B (**2**) (19.2 mg, 72%) was obtained as a white solid.

1-(3′,6′-Bis((*tert*-butyldiphenylsilyl)oxy)-2′-(hydroxymethyl)phenyl)heptyl Acetate (24). To a solution of **14** (32.4 mg, 0.040 mmol) in CH_2Cl_2 (2.00 mL, 0.02 M) were added acetic anhydride (7.5 μL, 0.0796 mmol), triethylamine (22.2 μL, 0.159 mmol), and *N*,*N*-dimethylpyridin-4-amine (1.0 mg, 0.00796 mmol) at rt. The reaction mixture was stirred for 12 h, quenched with saturated aqueous $NaHCO_3$, extracted with CH_2Cl_2 × 2 and EtOAc × 2, and dried over Na_2SO_4. The organic layer was filtrated and concentrated. The residue was purified by silica gel chromatography (*n*-hexane/EtOAc = 5:1). The crude product **23** was used for the following reaction without further purification.

To a solution of the crude product **23** in MeOH/THF (3.35 mL, v/v = 1:1, 0.01 M) was added *p*-toluenesulfonic acid monohydrate (9.6 mg, 0.0503 mmol) at 0 °C. The reaction mixture was stirred for 2 h at rt, quenched with saturated aqueous $NaHCO_3$, extracted with CH_2Cl_2 × 2 and EtOAc × 2, and dried over Na_2SO_4. The organic layer was filtrated and concentrated. The residue was purified by silica gel chromatography (*n*-hexane/EtOAc = 5:1). Compound **24** (20.4 mg, 66% in 2 steps) was obtained as a white solid.

1-(6′-((*tert*-Butyldiphenylsilyl)oxy)-3′-hydroxy-2′-(hydroxymethyl)phenyl)heptyl Acetate (13). To a solution of **24** (1.45 g, 1.70 mmol) in THF/pyridine (34.0 mL, v/v = 2:1, 0.050 M) was added hydrogen fluoride pyridine complex (1.0 mL) at 0 °C. The reaction mixture was stirred for 2 h, quenched with saturated aqueous $NaHCO_3$, extracted with EtOAc, and concentrated. Pyridine was removed by using azetrope with benzene, and the residue was purified by silica gel chromatography (*n*-hexane/EtOAc = 3:1). Compound **13** (851 mg, 94%) was obtained as a white solid.

1-(6′-((*tert*-Butyldiphenylsilyl)oxy)-2′,2′-dimethyl-4*H*-benzo[*d*][1′,3′]dioxin-5′-yl)heptyl Acetate (25). To a solution of **13** (851 mg, 1.59 mmol) in CH_2Cl_2 (53.1 mL, 0.03 M) were added 2,2-dimethoxypropane (0.49 mL, 3.98 mmol) and *p*-toluenesulfonic acid monohydrate (1.5 mg, 0.0796 mmol). The reaction mixture was stirred for 30 min, quenched with saturated aqueous $NaHCO_3$, extracted with EtOAc, and concentrated. The residue was purified by silica gel chromatography (*n*-hexane/EtOAc = 3:1). Compound **25** (838 mg, 92%) was obtained as a colorless oil.

5-(1′-Hydroxyheptyl)-2,2-dimethyl-4*H*-benzo[*d*][1,3]dioxin-6-ol (*rac*-7). To a solution of **25** (422 mg, 0.734 mmol) in THF (24.5 mL, 0.03 M) was added 1.0 M lithium aluminum hydride in THF (1.84 mL) at 0 °C. The reaction mixture was stirred for 5 min, quenched with MeOH and saturated aqueous Rochell's salt, extracted with EtOAc, and dried over Na_2SO_4. The organic layer was filtrated and concentrated. The residue was purified by silica gel chromatography (*n*-hexane/EtOAc = 3:1). Compound *rac*-7 (214 mg, 99%) was obtained as a white solid.

(*R*)-1-(6′-Hydroxy-2′,2′-dimethyl-4*H*-benzo[*d*][1′,3′]dioxin-5′-yl)heptyl 2,2-Diphenylacetate ((*R*)-26) and (*S*)-5-(1′-Hydroxyheptyl)-2,2-dimethyl-4*H*-benzo[*d*][1,3]dioxin-6-ol ((*S*)-7). To a solution of *rac*-7 (69.3 mg, 0.235 mmol) in Et_2O (2.3 mL, 0.1 M) were added *N*,*N*-diisopropylethylamine (0.030 mL, 0.169 mmol), (*R*)-benzotetramisole (3.0 mg, 0.0118 mmol), and diphenylacetic anhydride (57.0 mg, 0.141 mmol). The reaction mixture was stirred for 12 h, quenched with saturated aqueous $NaHCO_3$, extracted EtOAc, and dried over Na_2SO_4. The organic layer was filtrated and concentrated. The residue was purified by silica gel chromatography (*n*-hexane/EtOAc = 4:1, then CH_2Cl_2). Compound (*R*)-**26** (33.1 mg, 29%, 12% ee) was obtained as a colorless oil, and compound (*S*)-7 (37.1 mg, 54%, 50% ee) was obtained as a white solid.

(*S*)-5-(1′-Hydroxyheptyl)-2,2-dimethyl-4*H*-benzo[*d*][1,3]-dioxin-6-ol ((*S*)-7) (95% ee). To a solution of (*S*)-7 (37.1 mg, 0.126 mmol, 50% ee) in Et_2O (1.3 mL, 0.1 M) were added *N*,*N*-diisopropylethylamine (0.014 mL, 0.0339 mmol), (*R*)-benzotetramisole (1.6 mg, 0.00628 mmol), and diphenylacetic anhydride (13.8 mg, 0.0339 mmol). The reaction mixture was stirred for 12 h, quenched with saturated aqueous $NaHCO_3$, extracted with EtOAc, and dried over Na_2SO_4. The organic layer was filtrated and concentrated. The residue was purified by silica gel chromatography (*n*-hexane/EtOAc = 4:1, then CH_2Cl_2). Compound (*S*)-7 (35.9 mg, 97%, 54% ee) was obtained as a white solid. The chiral enriched (*S*)-7 (18.8 mg, 95% ee) was obtained by the same kinetic resolution after four repetitions from the above (*S*)-7 (35.9 mg, 0.126 mmol, 54% ee).

(*S*)-7 (95% ee): $[\alpha]_D^{25}$ −46.3 (*c* 1.12, $CHCl_3$). Other spectra are the same as those of *rac*-7.

(*S*)-2-(1′-Hydroxyheptyl)-3-(hydroxymethyl)benzene-1,4-diol ((*S*)-Violaceoid B, (*S*)-2)). To a solution of (*S*)-7 (69.5 mg, 0.236 mmol, 95% ee) in THF/H_2O (2.36 mL, v/v = 1:1, 0.1 M) was added *p*-toluenesulfonic acid monohydrate (22.4 mg, 0.118 mmol). The reaction mixture was stirred for 6 h, quenched with saturated aqueous $NaHCO_3$, extracted with EtOAc, and concentrated. The residue was purified by silica gel chromatography (*n*-hexane/EtOAc = 3:1) to afford (*S*)-violaceoid B (9.2 mg, 15%) as a white solid, and (*S*)-7 (59.6 mg, 86%) was recovered.

Additionally, (*S*)-violaceioid B (25.7 mg) and (*S*)-7 (27.1 mg) were obtained by the same reaction after two repetitions from the recovered (*S*)-7 (59.6 mg, 0.202 mmol). Therefore, 34.9 mg (total amount) of (*S*)-violaceoid (58%) was prepared from the starting 69.5 mg of (*S*)-7.

(*S*)-Violaceoid B ((*S*)-**2**) (95% ee): $[\alpha]_D^{23}$ −17.0 (*c* 0.093, $CHCl_3$), $[\alpha]_D^{23}$ −22.5 (*c* 0.667, MeOH). Other spectra are the same as those of *rac*-**2**.

(*S*)-1-(6′-Hydroxy-2′,2′-dimethyl-4*H*-benzo[*d*][1′,3′]dioxin-5′-yl)heptyl 2,2-diphenylacetate ((*S*)-26) and (*R*)-5-(1′-Hydroxyheptyl)-2,2-dimethyl-4*H*-benzo[*d*][1,3]dioxin-6-ol ((*R*)-7). To a solution of *rac*-7 (123.7 mg, 0.420 mmol) in Et_2O (4.2 mL, 0.1 M) were added *N*,*N*-diisopropylethylamine (0.050 mL, 0.303 mmol), (*S*)-benzotetramisole (5.0 mg, 0.0210 mmol), and diphenylacetic anhydride (102 mg, 0.252 mmol). The reaction mixture was stirred for 12 h, quenched with saturated aqueous $NaHCO_3$, extracted with EtOAc, and dried over Na_2SO_4. The organic layer was filtrated and concentrated. The residue was purified by silica gel chromatography (*n*-hexane/EtOAc = 4:1, then CH_2Cl_2). Compound (*S*)-**26** (65.9 mg, 32%, 19% ee) was obtained as a colorless oil, and compound (*R*)-7 (60.1 mg, 49%, 58% ee) was obtained as a white solid.

(*S*)-**26** (19% ee): $[\alpha]_D^{26}$ −0.20 (*c* 2.39, $CHCl_3$). Other spectra are the same as those of (*R*)-**26**.

(*R*)-5-(1′-Hydroxyheptyl)-2,2-dimethyl-4*H*-benzo[*d*][1,3]-dioxin-6-ol ((*R*)-7) (93% ee). To a solution of (*R*)-7 (60.1 mg, 0.204 mmol, 58% ee) in Et_2O (2.0 mL, 0.10 M) were added *N*,*N*-diisopropylethylamine (9.6 μL, 0.0550 mmol), (*S*)-benzotetramisole (2.6 mg, 0.0118 mmol), and diphenylacetic anhydride (22.3 mg, 0.0550 mmol). The reaction mixture was stirred for 12 h, quenched with saturated aqueous $NaHCO_3$, extracted with EtOAc, and dried over Na_2SO_4. The organic layer was filtrated and concentrated. The residue was purified by silica gel chromatography (*n*-hexane/EtOAc = 4:1, then CH_2Cl_2). Compound (*R*)-7 (56.8 mg, 95%, 66% ee) was obtained as a white solid. The chiral enriched (*R*)-7 (31.0 mg, 93% ee) was obtained by the same kinetic resolution after four repetitions from the above (*R*)-7 (56.8 mg, 0.193 mmol, 66% ee).

(*R*)-7 (93% ee): $[\alpha]_D^{25}$ +44.6 (*c* 1.87, $CHCl_3$). Other spectra are the same as those of *rac*-7.

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(*R*)-2-(1′-Hydroxyheptyl)-3-(hydroxymethyl)benzene-1,4-diol ((*R*)-Violaceoid B, (*R*)-2). To a solution of (*R*)-7 (25.1 mg, 0.0853 mmol, 93% ee) in THF/H_2O (1.71 mL, v/v = 1:1, 0.05 M) was added *p*-toluenesulfonic acid monohydrate (8.1 mg, 0.0426 mmol). The reaction mixture was stirred for 6 h, quenched with saturated aqueous $NaHCO_3$, extracted with EtOAc, and concentrated. The residue was purified by silica gel chromatography (*n*-hexane/EtOAc = 3:1) to afford (*R*)-violaceoid B (4.6 mg, 21%) as a white solid, and (*R*)-7 (19.6 mg, 78%) was recovered.

Additionally, (*R*)-violaceoid B (4.6 mg) and (*R*)-7 (12.2 mg) were obtained by the same reaction after two repetitions from the recovered (*R*)-7 (19.6 mg, 0.0618 mmol). Therefore, 9.2 mg (total amount) of (*R*)-violaceoid (54%) was prepared from the starting 25.1 mg of (*R*)-7.

(*R*)-Violaceoid B ((*R*)-**2**) (93% ee): $[\alpha]_D^{23}$ +15.9 (*c* 0.087, $CHCl_3$), $[\alpha]_D^{23}$ +22.9 (*c* 0.667, MeOH). Other spectra are the same as those of *rac*-**2**.

Biological Assay. Human breast cancer cell lines MCF-7 and Hs 578T were obtained from Japanese Collection of Research Bioresources (JCRB) Cell Bank (Osaka, Japan) and American Type Culture Collection (Manassas, VA, USA), respectively. Cells were cultured with Dulbecco's modified Eagle's medium supplemented with antibiotic−antimycotic (100 U/mL penicillin, 100 μg/mL streptomycin, 0.25 μg/mL amphotericin B; Thermo Fisher Scientific, Inc., Waltham, MA, USA), gentamicin (10 μg/mL, Thermo Fisher Scientific, Inc.), and 10% heat-inactivated fetal bovine serum. MCF-7 cells or Hs 578T cells were seeded in 96-well plates (1000 cells/well) and incubated for 48 h at 37 °C. Cells were then incubated with violaceoid A or (*S*)- and (*R*)-violaceoid B (10−100 μM) for 48 h, and cell number was estimated by WST-8 reagent (Cell Counting Kit-8, Dojindo Laboratories, Japan).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.8b00215.

Copies of ^1H and ^{13}C NMR spectra for all new compounds (**1**, **2**, **7**, and **12**−**26**), HPLC data of compounds *rac*-**7**, (*S*)-**7**, and (*R*)-**7**, and additional data (procedure for the reduction of ester **26** to the corresponding alcohol **7** and for the determination of the absolute configuration of chiral alcohols (*S*)-**7** and (*R*)-**7**) (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: shiina@rs.kagu.tus.ac.jp.

ORCID

Isamu Shiina: 0000-0002-8749-2540

Notes

The authors declare no competing financial interest.

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