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Total Synthesis of 4,5-Didehydroguadiscine: A Potent Melanogenesis Inhibitor from the Brazilian Medicinal Herb, *Hornschuchia obliqua*

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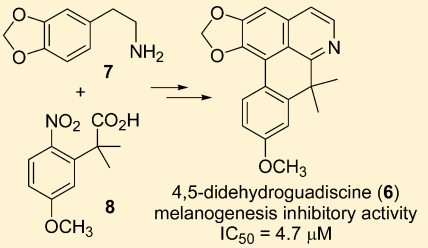
Supporting Information

ABSTRACT: The first total synthesis of the 7,7-dimethylaporphinoid, 4,5-didehydroguadiscine (**6**), originally isolated from the stems and roots of *Hornschuchia oblique* (Annonaceae), was achieved by the condensation of homopiperonylamine (**7**) with an α,α-dimethylphenylacetic acid derivative (**8**) and subsequent Pschorr reaction of the resulting benzylisoquinoline intermediate (**22**). The reported ^{13}C NMR data were partially revised on the basis of the analysis of HMBC spectra measured under different conditions. The melanogenesis inhibitory activity (IC_{50} = 4.7 μM) of **6** was 40 times stronger than that of arbutin (174 μM), which was used as reference standard. Furthermore, **6** was the most potent natural melanogenesis inhibitor within this class of compounds.



The aporphinoids are a diverse family of isoquinoline alkaloids including more than 300 members.[1] They share a characteristic tetracyclic motif with different levels of oxidation on both aromatic rings (Figure 1). A range of important biological activities has been documented, including serotonergic, antiplatelet, anticancer, antimalarial, and vaso-relaxing activities.[2] These properties have prompted intensive structure−activity relationship (SAR) studies over the past three decades.[3] Several aporphinoids have been isolated from the flower buds and leaves of the sacred lotus (*Nelumbo nucifera*, Nymphaeaceae), and some inhibit melanogenesis.[3a] Of these aporphinoids, four (**1**, **2**, **3**, and **4**) significantly inhibited melanogenesis (IC_{50} = 13.3−19.3 μM) in theophylline-stimulated murine B16 melanoma 4A5 cells, whereas a 4,5-didehydro type aporphinoid, lysicamine (**5**), was nearly inactive at the same concentration.[3a] During the course of our continued SAR studies on these aporphinoids and their derivatives, we achieved the first total synthesis of 4,5-didehydroguadiscine (**6**), a didehydroaporphinoid constituent originally isolated from the stems and roots of *Hornschuchia obliqua* (Annonaceae).[4] Intensive NMR spectroscopic studies on **6** including HMBC measurements under different conditions permitted the unambiguous assignment of the ^{13}C NMR signals and revision of the reported ^{13}C NMR data. The melanogenesis inhibitory activity of **6** was found ca. 40 times greater than that of arbutin, which was used as reference standard.

RESULTS AND DISCUSSION

On the basis of the retrosynthetic analysis of 4,5-didehydroguadiscine (**6**) (Scheme 1), the starting materials homopiperonylamine (**7**) and 2-methyl-2-(5-methoxy-2-nitrophenyl)propionic acid (**8**) were synthesized. According to the literature method,[5] homopiperonylamine (**7**) was first derived from piperonal (**9**) in good yield through the nitroaldol reaction with nitromethane followed by $LiAlH_4$ reduction of the corresponding nitroalkene (**10**). The propionic acid (**8**) was synthesized in the following manner. Commercially available 3-methyl-4-nitroanisole (**11**) was condensed with diethyl oxalate in the presence of ^{t}BuOK. Subsequent oxidative degradation of the corresponding pyruvate by alkaline H_2O_2 afforded (5-methoxy-2-nitrophenyl)acetic acid[6] (**12**) in 66% yield. Treatment of the acid with methanol under Fischer esterification conditions gave methyl (5-methoxy-2-nitrophenyl)acetate[6a,7] (**13**), which was subjected to a one-pot α,α-dimethylation in dry THF according to the protocol reported by Prasad and co-workers.[8] Treatment of **13** with 2.2 equiv of MeI and ^{t}BuOK in the presence of 18-Crown-6 resulted in the formation of a ca. 1.4:1 mixture of the target methyl 2-(5-methoxy-2-nitrophenyl)-2-methylpropionate[8] (**14**) and the α-monomethylated compound methyl 2-(5-methoxy-2-nitrophenyl)propionate[8,9] (**15**). The yield of α,α-dimethylation was improved by treating compound **13** with an excess of ^{t}BuOK (4 equiv) and MeI (5 equiv) to form **14** in 97% yield. Degassed solvent was essential for an optimal reaction. The α-oxygenated products methyl 2-hydroxy-2-(5-methoxy-2-nitrophenyl)propionate (**16**) and/or 2-methoxy-2-(5-methoxy-2-nitrophenyl)propionate (**17**) were formed as by-products unless the mixture of the reactants was degassed prior to ^{t}BuOK addition. Ester **14** was saponified with KOH in a mixture of H_2O and MeOH to form **8** in 96% yield (Scheme 2).

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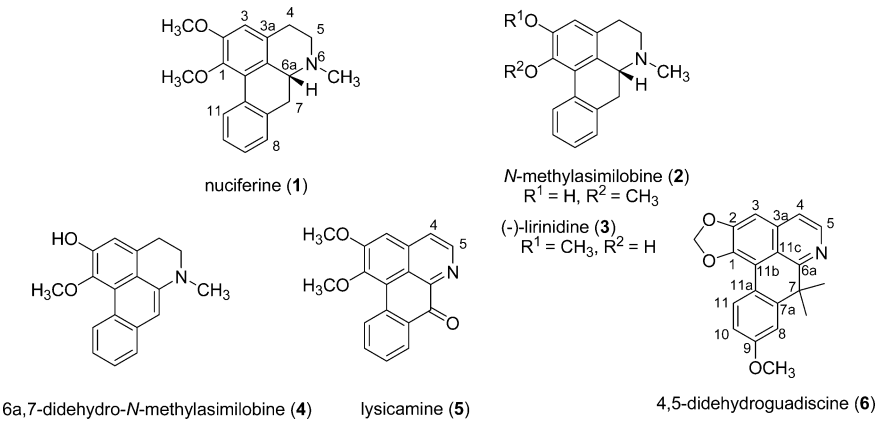
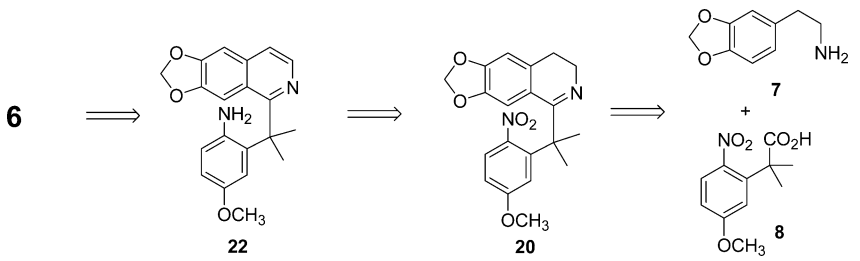
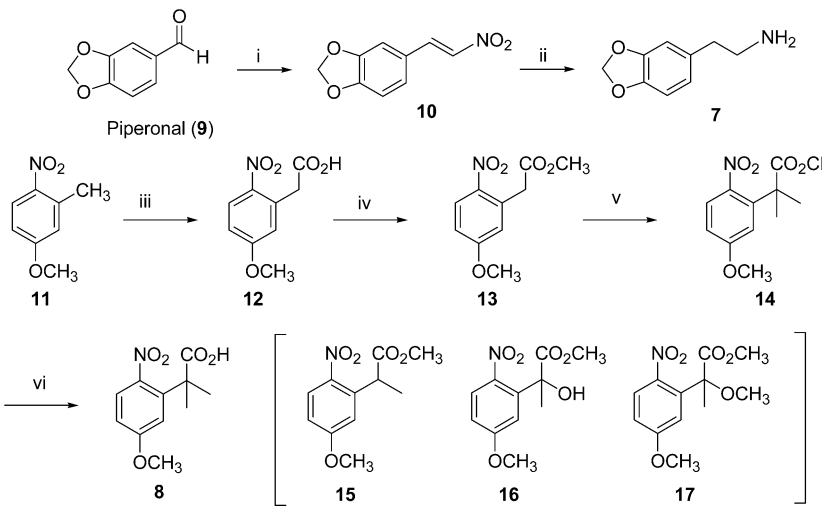


Figure 1. Structures of aporphinoids.

Scheme 1. Retrosynthesis of 4,5-Didehydroguadiscine (6)



Scheme 2. Syntheses of Building Blocks of 4,5-Didehydroguadiscine (6)[a]



[a]Reagents and conditions: (i) CH_3NO_2, NH_4OAc, HOAc, reflux, 2 h; (ii) $LiAlH_4$, THF, reflux, 4 h (71% from **9**); (iii) $(CO_2Et)_2$, ^tBuOK, Et_2O, rt, 30 min, then 10 N aq NaOH, H_2O_2, 0 °C → rt, 1 h (66%); (iv) MeOH, cat. concd H_2SO_4, reflux, 3 h (96%); (v) CH_3I, 18-Crown-6, ^tBuOK, degassed THF, −78 °C → rt (97%); (vi) KOH, MeOH-H_2O (1/1, v/v), reflux, 7 h (96%).

The coupling of compounds **7** and **8** via the acid chloride, 2-methyl-2-(5-methoxy-2-nitrophenyl)propionyl chloride (**18**), proceeded in poor yield (16%) of the amide, *N*-homopiperonyl-2-methyl-2-(5-methoxy-2-nitrophenyl)propionamide (**19**). Therefore, an effective peptide synthetic method developed by Carpino[10] was applied. Thus, treatment of a mixture of **7** and **8** with 1-hydroxy-7-azabenzotriazole (HOAt), 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (EDC·HCl) and 1,8-bis(*N*,*N*-dimethylamino)naphthalene (PS: proton sponge) at 70 °C for 3 h in DMF afforded **19** in 73% yield. The intramolecular cyclization reaction of **19** with $POCl_3$ in toluene and subsequent dehydrogenation of the dihydroisoquinoline derivative, 3,4-dihydro-1-[1,1-dimethyl-1-(5-methoxy-2-nitrophenyl)methyl]-6,7-methylenedioxyisoquinoline (**20**), by MnO_2 afforded 1-[1-methyl-1-(5-methoxy-2-nitrophenyl)ethyl]-6,7-methylenedioxyisoquinoline (**21**) from **19** in 88% yield. Both attempted catalytic hydrogenation of the nitro group in **21** over Pd−C and treatment of **21** with $LiAlH_4$ formed 1-[1-methyl-1-(5-methoxy-2-aminophenyl)ethyl]-6,7-methylenedioxyisoquinoline (**22**) in poor yields and were accompanied by poorly separable impurities. Thus, target amine **22** was obtained by zinc-mediated reduction in HOAc in 56% yield. The Pschorr cyclization of the diazonium salt (**23**) derived from the diazotization of **22** in the presence of MeOH

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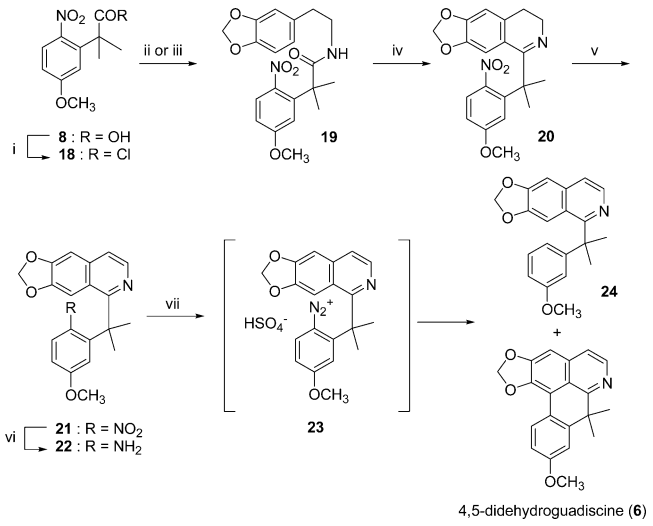
as a cosolvent[11] gave a mixture of 4,5-didehydroguadiscine (**6**) (30%) and 1-[1-methyl-1-(5-methoxyphenyl)ethyl]-6,7-methylenedioxyisoquinoline (**24**) (29%). The formation of **24** could be attributed to one-electron reduction of the diazonium function of **23** with CuCl followed by hydrogen abstraction of the resulting aryl radical from methanol.[12] Thus, the diazonium salt **23** was treated with CuCl under methanol-free conditions to exclusively obtain 4,5-didehydroguadiscine (**6**) in 58% yield (Scheme 3).

Scheme 3. Synthesis of 4,5-Didehydroguadiscine (6)[a]



[a]Reagents and conditions: (i) $SOCl_2$, benzene, reflux, 2.5 h; (ii) **7**, Et_3N, CH_2Cl_2, rt, 18 h (16% from **8**); (iii) EDC·HCl, HOAt, PS, DMF, **7**, 70 °C, 5 h (73%); (iv) $POCl_3$, toluene, reflux 3 h (98%); (v) MnO_2, toluene, reflux 7.5 h (90%); (vi) Zn, NH_4Cl, H_2O, rt, 2 h (56%); (vii) $NaNO_2$, 10% H_2SO_4, H_2O, 0 °C, 1 h, then CuCl, 50 °C, 4 h (58%).

The ^{1}H NMR data of synthetic **6** agreed well with those for the natural species[4] (with deviations $\Delta\delta_H < 0.04$ ppm, Table 1). However, significant deviations ($\Delta\delta_c = -8.1 - +0.9$ ppm) were observed in the ^{13}C NMR data of the reported and our synthetic sample (Table 2). Analysis of the HMBC spectroscopic data led to revision of the reported chemical shift of C-1 (δ_c 143.0) to δ_c 151.1 on the basis of the HMBC correlation (C−H coupling constant, 8 Hz) of C-1 (δ_C 151.1) and H3 (δ_H 7.01). No correlation was observed between these two signals in the HMBC spectrum measured at a 35 Hz coupling constant. Similarly, the signal at δ_C 118.4 assigned to C-11b has to be revised to δ_C 112.4, which correlates with H-11 (δ_H 8.51) in the HMBC spectrum measured at 8 Hz, whereas no correlation was observed between these signals in the spectrum measured at 35 Hz. Thus, the δ_C 151.1 and 112.4 resonances were unambiguously assigned to C-1 and C-11b, respectively.

Finally, the inhibitory effect of 4,5-didehydroguadiscine (**6**) on melanogenesis was tested[13] and compared to the related aporphinoids and arbutin. 4,5-Didehydroguadiscine (**6**, IC_{50} = 4.7 μM) exhibited inhibitory activity superior to any related natural aporphinoids (**1**−**4**, IC_{50} = 13.3−19.3 μM[3a]) (Table 3). Compound **6** is approximately 40 times as potent as arbutin (IC_{50} = 174 μM), which was used as reference standard. It is also noteworthy that compound **6** is more potent than lysicamine (**5**),[3a] a representative of the 4,5-didehydroaporphinoid family. No significant cytotoxicity was observed at the effective concentrations (95.7 ± 9.3% viability at 3 μM, 86.7 ± 8.1% at 10 μM). Thus, **6** is the most potent natural melanogenesis inhibitor within this class of compounds. Further SAR studies addressing the melanogenesis inhibitory activity and origin of the cytotoxicity of aporphinoids and related compounds are in progress.

EXPERIMENTAL SECTION

General Experimental Procedures. Melting points were determined on an AS ONE ATM-02 micromelting point apparatus and are uncorrected. IR spectra were measured on an IRAffinity-1 spectrophotometer. NMR spectra were recorded on a JEOL JNM-ECA 400 (400 MHz ^{1}H, 100 MHz ^{13}C), a JEOL JNM-ECA 500 (500 MHz ^{1}H, 125 MHz ^{13}C), or a JEOL JNM-ECA 800 (800 MHz ^{1}H, 200 MHz ^{13}C) instrument. Chemical shifts (δ) and coupling constants (J) are given in ppm and Hz, respectively. Low-resolution and high-resolution mass spectra were recorded on a JEOL JMS-700T spectrometer. Column chromatography was performed over Fuji Silysia silica gel BW-200. All organic extracts were dried over anhydrous Na_2SO_4 prior to evaporation.

Synthesis of Homopiperonylamine (7). According to the literature procedure,[5] a mixture of piperonal (**9**, 3.0 g, 20 mmol), nitromethane (3.2 mL, 60 mmol), NH_4OAc (1.5 g, 30 mmol), and HOAc (15 mL) was heated under reflux for 2 h. The reaction mixture was allowed to cool to room temperature, and the precipitate of short yellow needles (**10**, 2.7 g, 70%) was collected by filtration and washed with MeOH. The combined filtrate and washings were neutralized by addition of $NaHCO_3$, and the resulting mixture was extracted with EtOAc. The extract was washed with brine and the solvent evaporated in vacuo to give a pale brown solid (1.1 g), which on trituration with MeOH gave **10** (200 mg, 5%) as a yellow solid.

Table 1. ^{1}H NMR Spectroscopic Data for 4,5-Didehydroguadiscine (6) in $CDCl_3$ (δ in ppm)

position	reported[4] (500 MHz) δ_H (J in Hz)	observed (800 MHz) δ_H (J in Hz)	$\Delta\delta_H$[c]	HMBC[a] correlations	HMBC[b] correlations
3	7.01 s	6.98 s	0.03	C-1, C-2, C-11c	C-2, C-11c
4	7.35 d (5.5)	7.32 d (5.6)	0.03	C-3, C-5, C-11c	–
5	8.44 d (5.5)	8.43 d (5.6)	0.01	C-3a, C-4, C-6a	C-3a, C-4, C-6a
8	7.24 d (2.9)	7.21 d (3.2)	0.03	C-7, C-9, C10, C-11a	C-7, C-9, C10, C-11a
10	6.95 dd (8.9, 2.9)	6.92 dd (8.8, 3.2)	0.03	C-8, C11a	C11a
11	8.52 d (8.8)	8.51 d (8.8)	0.01	C7a, C-9, C-11b	C7a
$C(CH_3)_2$	1.81 s	1.78 s	0.03	C-6a, C-7, C-7a	C-6a, C-7, C-7a
OCH_3	3.93 s	3.91 s	0.02	C-9	C-9
OCH_2O	6.24 s	6.20 s	0.04	C-1, C-2	–

[a,b]Measured under the long-range J_{C-H} values of 8 Hz[a] and 35 Hz[b]. [c]Deviations of the chemical shifts between those reported[4] and those observed in the present study.

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Table 2. ^{13}C NMR Spectroscopic Data for 4,5-Didehydroguadiscine (6) in $CDCl_3$ (δ in ppm)

position	reported[4] (125 MHz) δ_C	observed (200 MHz) δ_C	$\Delta\delta_C$[a]	position	reported[4] (125 MHz) δ_C	observed (200 MHz) δ_C	$\Delta\delta_C$[a]
1	143.0	151.1	−8.1	9	161.1	159.7	0.4
2	142.6	142.3	0.3	10	112.3	111.9	0.6
3	101.4	100.9	0.5	11	129.4	128.8	0.6
3a	135.5	134.6	0.9	11a	121.0	120.4	0.6
4	118.8	118.2	0.6	11b	118.4	112.4	6.0
5	142.8	142.4	0.4	11c	118.7	118.7	0
6a	162.3	162.5	−0.2	$C(CH_3)_2$	33.5	33.2	0.3
7	43.0	42.3	−0.3	OCH_3	55.6	55.3	0.3
7a	147.8	147.3	0.5	OCH_2O	101.6	101.1	0.5
8	113.2	112.8	0.4				

[a]Deviations of the chemical shifts between those reported[4] and those observed in the present study.

Table 3. Inhibitory Activity of Aporphinoids 1, 2, 3, 4, 5, 6 and Arbutin against Theophylline-Stimulated Melanogenesis in B16 Melanoma 4A5 Cells

	1[a]	2[a]	3[a]	4[a]	5[a]	6	Arbutin
IC_{50} (μM)	15.8	14.5	19.3	13.3	–	4.7	174

[a]See literature (ref 3a).

A solution of **10** (1.6 g, 8.3 mmol) in dry THF (10 mL) was added dropwise to a suspension of $LiAlH_4$ (900 mg, 24 mmol) in dry THF (30 mL) at 0 °C. After the mixture was heated under reflux for 4 h, the excess hydride was decomposed successively with EtOAc and 10% aqueous NaOH. The resulting mixture was filtered through Celite, and the residue was washed with Et_2O. The combined filtrate and washings were extracted with Et_2O. The extract was washed with brine, and the solvent was evaporated to give title compound **7** (1.3 g, 95%) as a pale orange semisolid, which was used in the next step without further purification. ^1H NMR (400 MHz, $CDCl_3$) δ: 2.67 (2H, t, J = 6.8, $CH_2CH_2NH_2$), 2.91 (2H, t, J = 6.8, $CH_2CH_2NH_2$), 5.92 (2H, s, OCH_2O), 6.62−6.76 (3H, m, arom.). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 39.6 ($CH_2CH_2NH_2$), 43.5 ($CH_2CH_2NH_2$), 100.7 (OCH_2O), 108.1/109.0/121.6 (d, arom.), 133.4/145.8/147.6 (s, arom.).

Synthesis of (5-Methoxy-2-nitrophenyl)acetic Acid (12). To a mixture of 3-methyl-4-nitroanisole (**11**, 3.0 g, 18.0 mmol), diethyl oxalate (4.9 mL, 36.1 mmol), and dry Et_2O (20 mL), ^{t}BuOK (3.0 g, 26.7 mmol) was added at room temperature. After the mixture was stirred at room temperature for 30 min, the reaction was quenched by adding cold H_2O (50 mL). To the resulting mixture, 10 N aqueous NaOH (5 mL) and a 30% aqueous solution of H_2O_2 (5 mL) were successively added with ice cooling. After the exothermic reaction subsided, the mixture was stirred at room temperature for 1 h. The resulting orange suspension was filtered, and the filtrate was washed with EtOAc. Na_2SO_3 was added to the aqueous layer until a negative test to starch iodide paper was obtained, and the resulting mixture was acidified with concd HCl to pH 2. The deposited pale yellow solid was collected by filtration and washed with H_2O to give title compound **12** (2.5 g, 66%) as a pale yellow solid, which was recrystallized from MeOH to give **12** as slightly yellowish prisms: mp, 182−183 °C [lit.[6a] 177 °C; lit.,[6b] 174−176 °C, lit.[6c] 175−177 °C]. ^1H NMR (400 MHz, DMSO-d_6) δ: 3.86 (3H, s, OCH_3), 3.98 (2H, s, H-2), 7.05 (1H, dd, J = 9.0, 2.8, phenyl H-4), 7.09 (1H, d, J = 2.8, phenyl H-6), 8.13 (1H, d, J = 9.0, phenyl H-3), 12.5 (1H, br s, CO_2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 39.8 (C-2), 56.2 (OCH_3), 113.2 (phenyl C-4), 118.8 (phenyl C-6), 127.6 (phenyl C-3), 133.8 (phenyl C-1), 141.4 (phenyl C-2), 163.1 (phenyl C-5), 171.3 (CO_2H).

Synthesis of Methyl (5-Methoxy-2-nitrophenyl)acetate (13). A mixture of **12** (2.26 g, 4.7 mmol), concd H_2SO_4 (0.3 mL), and MeOH (60 mL) was heated under reflux for 3 h. After cooling, the reaction mixture was neutralized with $NaHCO_3$. The resulting suspension was filtered, and the filtrate was concentrated in vacuo to give a brown semisolid (3.37 g), which was triturated with $CHCl_3$ (2 × 15 mL and 1 × 5 mL), and the $CHCl_3$ solution was condensed in vacuo. The residue (2.45 g) was purified by column chromatography (*n*-hexane-EtOAc, 30:1 → 10:1 → 3:1) to give compound[6a,7] **13** (2.31 g, 96%) as a slightly yellowish microcrystalline solid: mp, 64−65 °C; lit.,[6a] 66 °C. ^1H NMR (400 MHz, $CDCl_3$) δ: 3.71 (3H, s, CO_2CH_3), 3.90 [3H, s, $C_6H_3(NO_2)OCH_3$], 4.01 (2H, s, H-2), 6.78 (1H, d, J = 2.8, phenyl H-6), 6.91 (1H, dd, J = 9.2, 2.8, phenyl H-4), 8.20 (1H, d, J = 9.2, phenyl H-3). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 40.4 (C-2), 52.2 (CO_2CH_3), 55.9 (OCH_3), 113.0 (phenyl C-4), 118.6 (phenyl C-6), 128.0 (phenyl C-3), 132.7 (phenyl C-1), 141.5 (phenyl C-2), 163.4 (phenyl C-5), 170.4 (CO_2CH_3).

***α,α*-Dimethylation of Compound 13.** A mixture of **13** (1.5 g, 6.67 mmol), freshly distilled MeI (2.1 mL, 34 mmol), 18-Crown-6 (440 mg, 1.67 mmol), and dry THF (90 mL) was degassed by ultrasonication prior to the addition of ^{t}BuOK. Under an Ar atmosphere, the mixture was cooled to −78 °C, and ^{t}BuOK (2.98 g, 26.7 mmol) was added with stirring. The resulting suspension was stirred at −78 °C for 1 h and allowed to reach room temperature. After it was stirred at room temperature for 1 h, the reaction mixture was poured into cold aqueous NH_4Cl (100 mL) and extracted with EtOAc (3 × 50 mL). The extract was washed with brine and the solvent evaporated in vacuo to give a pale yellow solid (1.69 g), which on column chromatography ($CHCl_3$) gave methyl 2-(5-methoxy-2-nitrophenyl)-2-methylpropionate[7,8] (**14**, 1.64 g, 97%).

In contrast, when the reaction was performed using the reported molar ratio,[8] a mixture of **13** (225 mg, 1.0 mmol), MeI (0.14 mL, 2.2 mmol), 18-Crown-6 (66 mg, 0.25 mmol), degassed dry THF (15 mL), and ^{t}BuOK (246 mg, 2.2 mmol) gave a mixture of *α,α*-dimethylated ester **14** and the *α*-monomethylated ester, methyl (5-methoxy-2-nitrophenyl)propionate[9] (**15**), as a pale yellow oil (248−268 mg, **14**/**15** = ca. 1.3−1.5/1).

The formation of a small amount of the byproducts methyl 2-hydroxy-2-(5-methoxy-2-nitrophenyl)propionate (**16**) and/or methyl 2-methoxy-2-(5-methoxy-2-nitrophenyl)propionate (**17**) was detected when the reaction was performed without ultrasonication prior to the addition of ^{t}BuOK.

*α,α-Dimethyl Ester (**14**).* A slightly yellowish microcrystalline solid: mp, 63−64 °C; lit.,[8] 61−62 °C. IR (KBr): 2985, 2951, 1721, 1609, 1578, 1508, 1473, 1338, 1319, 1273, 1249, 1153, 1135, 1061, 1037 cm^{-1}. ^1H NMR (800 MHz, $CDCl_3$) δ: 1.65 [6H, s, $C(CH_3)_2$], 3.65 (3H, s, CO_2CH_3), 3.91 [3H, s, $C_6H_3(NO_2)OCH_3$], 6.85 (1H, dd, J = 8.8, 2.4, phenyl H-4), 7.06 (1H, d, J = 2.4, phenyl H-6), 8.10 (1H, d, J = 8.8, phenyl H-3). ^{13}C NMR (200 MHz, $CDCl_3$) δ: 27.2 [$C(CH_3)_2$], 46.6 [$C(CH_3)_2$], 52.0 (CO_2CH_3), 55.7 [$C_6H_3(NO_2)OCH_3$], 110.6 (phenyl C-4), 114.9 (phenyl C-6), 128.6 (phenyl C-3), 141.5 (phenyl C-2), 142.4 (phenyl C-1), 163.2 (phenyl C-5), 175.6 (CO_2CH_3). FABMS (pos.) m/z: 254 $[M + H]^+$, 276 $[M + Na]^+$. FABHRMS m/z: 276.0875 (calcd for $C_{12}H_{15}O_5NNa$, 276.0848).

*α-Monomethyl Ester (**15**).* Colorless prisms (from *n*-hexane): mp, 58−59 °C; lit.,[9] mp, 60−62 °C. IR (KBr): 2951, 2845, 1372, 1612, 1582, 1508, 1477, 1462, 1438, 1339, 1315, 1296, 1242, 1120, 1172, 1076, 1010 cm^{-1}. ^1H NMR (800 MHz, $CDCl_3$) δ: 1.59 (3H, d, J = 7.2,

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$CHCH_3$), 3.68 (3H, s, CO_2CH_3), 3.89 [3H, s, $C_6H_3(NO_2)OCH_3$], 4.42 (1H, q, J = 7.2, $CHCH_3$), 6.87 (1H, dd, J = 8.8, 3.2, phenyl H-4), 6.91 (1H, d, J = 3.2, phenyl H-6), 8.07 (1H, d, J = 8.8, phenyl H-3). ^{13}C NMR (200 MHz, $CDCl_3$) δ: 17.6 ($CHCH_3$), 41.9 ($CHCH_3$), 52.2 (CO_2CH_3), 55.8 [$C_6H_3(NO_2)OCH_3$], 112.2 (phenyl C-4), 115.3 (phenyl C-6), 127.9 (phenyl C-3), 138.3 (phenyl C-1), 141.6 (phenyl C-2), 163.3 (phenyl C-5), 173.6 (CO_2CH_3). FABMS (pos.) m/z: 240 $[M + H]^+$.

α-Hydroxy Ester (16). Colorless prisms (from *n*-hexane-EtOAc): mp, 85−86 °C. IR (KBr): 3503, 3287, 3201, 2947, 2846, 1720, 1612, 1581, 1508, 1454, 1342, 1323, 1292, 1245, 1200, 1110, 1049, 1033 cm^{-1}. ^{1}H NMR (800 MHz, $CDCl_3$) δ: 1.86 [3H, s, $C(OH)CH_3$], 3.77 (3H, s, CO_2CH_3), 3.79 (1H, s, OH), 3.92 [3H, s, $C_6H_3(NO_2)OCH_3$], 6.90 (1H, dd, J = 8.8, 3.2, phenyl H-4), 7.24 (1H, d, J = 3.2, phenyl H-6), 7.99 (1H, d, J = 8.8, phenyl H-3). ^{13}C NMR (200 MHz, $CDCl_3$) δ: 26.7 [$C(OH)CH_3$], 53.0 (CO_2CH_3), 55.9 [$C_6H_3(NO_2)OCH_3$], 75.0 [$C(OCH_3)CH_3$], 112.1 (phenyl C-4), 115.2 (phenyl C-6), 128.1 (phenyl C-3), 139.7 (phenyl C-1), 141.1 (phenyl C-2), 163.1 (phenyl C-1), 174.3 (CO_2CH_3). FABMS (pos.) m/z: 256 $[M + H]^+$, 278 $[M + Na]^+$. FABHRMS m/z: 278.0647 (calcd for $C_{12}H_{15}O_6NNa$, 278.0641).

α-Methoxy Ester (17). Colorless prisms (from Et_2O): mp, 77−78 °C. IR (KBr): 2974, 2843, 1612, 1578, 1519, 1458, 1350, 1269, 1195, 1126, 1103, 1045 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.85 [3H, s, $C(OCH_3)CH_3$], 3.28 [3H, s, $C(OCH_3)CH_3$], 3.72 (3H, s, CO_2CH_3), 3.92 [3H, s, $C_6H_3(NO_2)OCH_3$], 6.91 (1H, dd, J = 8.9, 2.6, phenyl H-4), 7.35 (1H, d, J = 2.6, phenyl H-6), 8.06 (1H, d, J = 8.9, phenyl H-3). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 24.1 [$C(OCH_3)CH_3$], 52.2 (CO_2CH_3), 52.7 [$C(OCH_3)CH_3$], 55.9 [$C_6H_3(NO_2)OCH_3$], 81.3 [$C(OCH_3)CH_3$], 112.7 (phenyl C-4), 113.7 (phenyl C-6), 128.1 (phenyl C-3), 140.0 (phenyl C-1), 141.0 (phenyl C-2), 163.5 (phenyl C-1), 169.1 (CO_2CH_3). FABMS (pos.) m/z: 270 $[M + H]^+$, 292 $[M + Na]^+$. FABHRMS m/z: 292.0793 (calcd for $C_{12}H_{15}O_6NNa$, 292.0797).

Synthesis of 2-Methyl-2-(5-methoxy-2-nitrophenyl)-propionic Acid (8). A mixture of **14** (1.5 g, 5.92 mmol), KOH (3.3 g, 58.9 mmol), MeOH (6.0 mL), and H_2O (6.0 mL) was heated under reflux for 7 h. After the mixture was cooled, the reaction mixture was acidified with 10% HCl (pH ca. 3) and then extracted with EtOAc (3 × 30 mL). The extract was washed with brine and the solvent evaporated in vacuo. The residue (1.43 g) was purified by column chromatography ($CHCl_3$ → $CHCl_3$-MeOH, 50:1) to give compound **8** (1.36 g, 96%) as a pale brown solid. Recrystallization from a mixture of *n*-hexane−EtOAc gave **8** as slightly yellowish needles: mp, 190−191 °C. IR (KBr): 3448, 2978, 2939, 2650, 2526, 1717, 1581, 1504, 1342, 1319, 1289, 1262, 1195, 1084 cm^{-1}. ^{1}H NMR (800 MHz, $CDCl_3$) δ: 1.68 [$C(CH_3)_2$], 3.91 (3H, s, OCH_3), 6.85 (1H, dd, J = 9.6, 3.2 phenyl H-4), 7.07 (1H, d, J = 3.2, phenyl H-6), 8.15 (1H, d, J = 9.6, phenyl H-3), 12.5 (1H, br s, CO_2H). ^{13}C NMR (200 MHz, $CDCl_3$) δ: 27.0 [$C(CH_3)_2$], 46.6 [$C(CH_3)_2$], 55.8 (OCH_3), 110.9 (phenyl C-4), 115.0 (phenyl C-6), 128.9 (phenyl C-3), 141.2 (phenyl C-2), 142.0 (phenyl C1), 163.5 (phenyl C-5), 180.3 (CO_2H). FABMS (pos.) m/z: 240 $[M + H]^+$.

Synthesis of *N*-Homopiperonyl-2-methyl-2-(5-methoxy-2-nitrophenyl)propionamide (19). A mixture of amine **8** (1.28 g, 7.8 mmol), acid **9** (1.56 g, 6.5 mmol), 1-hydroxy-7-azabenzotriazole (HOAt, 980 mg, 7.2 mmol), 1-ethyl-3-(3-dimethylamonopropyl)-carbodiimide hydrochloride (EDC·HCl, 7.2 mmol), 1,8-bis(*N*,*N*-dimethylamino)naphthalene (PS, 710 mg, 3.3 mmol), and DMF (20 mL) was heated at 70 °C for 5 h. The reaction mixture was poured into cold H_2O (50 mL) and extracted with EtOAc (3 × 50 mL). The extract was successively washed with 10% HCl, aqueous $NaHCO_3$, and brine and the solvent evaporated in vacuo. The residue (2.51 g) was purified by column chromatography (*n*-hexane-EtOAc, 10:1 → 5:1 → 3:1) to give title compound **19** (1.84 g, 73%) as a pale yellow microcrystalline solid: mp, 116−117 °C. IR (KBr): 3379, 2932, 2882, 1636, 1520, 1354, 1284, 1250, 1180, 1068, 1041 cm^{-1}. ^{1}H NMR (800 MHz, $CDCl_3$) δ: 1.58 [6H, s, $C(CH_3)_2$], 2.71 (2H, t, J = 7.2, CH_2CH_2NHCO), 3.43 (2H, t, d, J = 7.2, 6.4, CH_2CH_2NHCO), 3.90 (3H, s, OCH_3), 5.34, (1H, br t, J = 6.4 N*H*), 5.92 (2H, s, OCH_2O), 6.57 (1H, dd, J = 8.0, 1.6, arom.), 6.63 (1H, d, J = 1.6, arom.), 6.68 (1H, d, J = 8.0, arom.), 6.84 (1H, dd, J = 8.8, 2.4, arom.), 7.05 (1H, d, J = 2.4, arom.), 7.98 (1H, d, J = 8.8, arom.). ^{13}C NMR (200 MHz, $CDCl_3$) δ: 27.4 [$C(CH_3)_2$], 35.2 (CH_2CH_2NHCO), 40.9 (CH_2CH_2NHCO), 47.1 [$C(CH_3)_2$], 55.8 (OCH_3), 100.8 (OCH_2O), 108.2/109.1/110.8/115.6/121.6/128.7 (d, arom.), 132.8/141.9/142.4/146.0/147.7/163.0 (s, arom), 175.2 (CONH). FABMS (pos.) m/z: 387 $[M + H]^+$.

Synthesis of 1-[1-Methyl-1-(5-methoxy-2-nitrophenyl)-ethyl]-6,7-methylenedioxyisoquinoline (21). To a solution of **19** (850 mg, 2.2 mmol) in dry toluene (20 mL), $POCl_3$ (1.0 mL, 11 mmol) was added at room temperature, and the mixture was heated under reflux for 3 h. After it was cooled, the reaction was quenched with H_2O (50 mL), and the pH of the resulting mixture was adjusted to ca. 10 with 10% NaOH and extracted with EtOAc (3 × 30 mL). The extract was washed with brine and the solvent evaporated in vacuo to give 3,4-dihydro-1-[1-methyl-1-(5-methoxy-2-nitrophenyl)ethyl]-6,7-methylenedioxyisoquinoline (**20**, 794 mg) as a pale brown solid, which was sufficiently pure for the subsequent reaction.

To a solution of **20** (300 mg, 0.82 mmol) in toluene (20 mL), MnO_2 (704 mg, 8.1 mmol) was added, and the resulting suspension was heated under reflux for 7 h. The reaction mixture was filtered by suction, and the filter cake was washed with EtOAc. The combined filtrate and washings were condensed in vacuo to give the title compound **21** (268 mg, 88% from **19**) as a pale brown solid, which was recrystallized from MeOH to give slightly yellowish prisms: mp, 166−167 °C. IR (KBr): 2985, 2912, 1609, 1573, 1519, 1466, 1431, 1350, 1242, 1037 cm^{-1}. ^{1}H NMR (800 MHz, $CDCl_3$) δ: 1.98 [6H, s, $C(CH_3)_2$], 3.90 (3H, s, OCH_3), 5.96 (2H, s, OCH_2O), 6.64 (1H, s, arom.), 6.82 (1H, dd, J = 8.8, 2.4, arom.), 7.01 (1H, s, arom.), 7.23 (1H, d, J = 2.4, arom.), 7.37 (1H, d, J = 5.6, arom.), 7.45 (1H, d, J = 8.8, arom.), 8.39 (1H, d, J = 5.6, arom.). ^{13}C NMR (200 MHz, $CDCl_3$) δ: 30.7 [$C(CH_3)_2$], 47.2 [$C(CH_3)_2$], 55.7 (OCH_3), 101.3 (OCH_2O), 102.2/103.6/110.8/114.1/119.8/127.1/140.3 (d, arom.), 122.3/135.6/144.1/144.6/147.3/149.4/161.7/162.0 (s, arom). FABMS (pos.) m/z: 367 $[M + H]^+$.

Synthesis of 1-[1-Methyl-1-(5-methoxy-2-aminophenyl)-ethyl]-6,7-methylenedioxyisoquinoline (22). To a solution of **21** (250 mg, 0.74 mmol) in MeOH (20 mL), zinc dust (446 mg, 6.8 mmol) and NH_4Cl (547 mg, 10.2 mmol) were successively added, and the resulting suspension was stirred at room temperature for 2 h. The reaction mixture was filtered by suction, and the filter cake was washed with MeOH. The combined filtrate and washings were condensed in vacuo. The residue (420 mg) was purified by column chromatography (*n*-hexane-EtOAc, 20:1 → 10:1 → 5:1) to give the title compound **22** (130 mg, 56%) as a slightly yellowish microcrystalline solid: mp, 155−157 °C. IR (KBr): 3453, 3333, 3225, 2986, 2913, 1640, 1620, 1578, 1501, 1466, 1427, 1281, 1242, 1041 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.84 [6H, s, $C(CH_3)_2$], 2.75 (2H, br s, NH_2), 3.84 (3H, s, OCH_3), 5.94 (2H, s, CH_2O_2), 6.40 (1H, d, J = 8.8, arom.), 6.66 (1H, dd, J = 8.8, 2.8, arom.), 7.00 (1H, s, arom.), 7.22 (1H, s, arom.), 7.24 (1H, d, J = 2.8, arom.), 7.39 (1H, d, J = 5.6, arom.), 8.37 (1H, d, J = 5.6, arom.). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 28.7 [$C(CH_3)_2$], 46.4[$C(CH_3)_2$], 55.8 (OCH_3), 101.3 (OCH_2O), 102.7/103.3/111.9/112.2/118.1/120.0/140.3 (d, arom.), 123.4/134.1/135.5/138.2/147.7/149.8/153.0/164.5 (s, arom.). FABMS (pos.) m/z: 337 $[M + H]^+$.

Synthesis of 4,5-Didehydroguadiscine (6). To a solution of **22** (55 mg, 0.15 mmol) in a mixture of H_2O (1.7 mL) and 10% H_2SO_4 (0.6 mL), $NaNO_2$ (21 mg, 0.3 mmol) was added at 0 °C, and the mixture was stirred at 0 °C for 1 h. The reaction mixture containing the diazonium salt **23** was heated at 50 °C for another 4 h after the addition of CuCl (15 mg, 0.15 mmol). The pH of the reaction mixture was adjusted to ca. 8 by $NaHCO_3$ and extracted with EtOAc. The extract was washed with brine and the solvent evaporated in vacuo. The residue (44 mg) was purified by column chromatography (*n*-hexane-EtOAc, 50:1 → 30:1) to give 4,5-didehydroguadiscine (**6**, 27.5 mg, 58%).

When a solution of diazonium salt **23** (prepared by the diazotization of **22** (70 mg, 0.21 mmol) with $NaNO_2$ (47 mg, 0.41 mmol) in a mixture of H_2O (2.4 mL), MeOH (3.2 mL), and 10% H_2SO_4 (0.8 mL) at 0 °C) was treated with CuCl (21 mg, 0.21 mmol), **6** (19.6 mg, 30%)

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was obtained together with 1-[1-methyl-1-(5-methoxyphenyl)ethyl]-6,7-methylenedioxyisoquinoline (**24**, 19.7 mg, 29%).

*4,5-Didehydroguadiscine (**6**).* Colorless needles (from MeOH): mp, 160−161 °C; lit.,[4] brownish amorphous powder, melting point was not reported. IR (KBr): 2967, 2928, 1609, 1570, 1512, 1501,1450, 1420, 1377, 1354, 1315, 1296, 1250, 1219, 1049 cm^{-1}. FABMS (pos.) *m/z*: 320 $[M + H]^+$. FABHRMS *m/z*: 320.1279 (calcd for $C_{20}H_{18}O_3N$, 320.1287). ^1H and ^{13}C NMR spectra of **6** are summarized in Table 1 and 2.

*Compound (**24**).* Colorless plates: mp, 109−110 °C. IR (KBr): 2974, 2908, 1601, 1578, 1465, 1461, 1257, 1211, 1041 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ: 1.81 [6H, s, $C(CH_3)_2$], 3.72 (3H, s, OCH_3), 5.94 (2H, s, OCH_2O), 6.72 (1H, ddd, *J* = 8.0, 2.6, 0.9, arom.), 6.77 (1H, dd-like, *J* = 2.6, 2.2, arom.), 6.79 (1H, ddd, *J* = 8.0, 2.2, 0.9, arom.), 6.92 (1H, s, H-8), 7.00 (1H, s, H-5), 7.19 (1H, dd, *J* = 8.0, 8.0, arom.), 7.34 (1H, d, *J* = 5.5, H-4), 8.38 (1H, d, *J* = 5.5, H-3). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 30.9 [$C(CH_3)_2$], 47.4 [$C(CH_3)_2$], 55.1 (OCH_3), 101.2 (OCH_2O), 103.2/104.1/110.4/112.3/118.4/119.8/129.6/140.0 (d, arom.), 123.2/135.7/147.0/149.3/152.2/159.8/164.4 (s, arom.). FABMS (pos.) *m/z*: 322 $[M + H]^+$. FABHRMS *m/z*: 322.1430 (calcd for $C_{20}H_{20}O_3N$, 322.1443).

Reagents for Bioassay. Dulbecco's modified Eagle's medium (DMEM, 4.5 g/L glucose) was purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.); fetal bovine serum (FBS), penicillin, and streptomycin were purchased from Gibco (Invitrogen, Carlsbad, CA, U.S.A.); and the other chemicals were purchased from Wako Pure Chemical Co., Ltd. (Osaka, Japan). The 48-well multiplate and 96-well microplate (Sumilon) were purchased from Sumitomo Bakelite Co., Ltd. (Tokyo, Japan).

Cell Culture. Murine B16 melanoma 4A5 cells (RCB0557)[14] were obtained from Riken Cell Bank (Tsukuba, Japan), and the cells were grown in DMEM supplemented with 10% FBS, penicillin G (100 units/mL), and streptomycin (100 μg/mL) at 37 °C in 5% CO_2/air. The cells were harvested by incubation in phosphate-buffered saline (−) [PBS (−)] containing 0.05% (w/v) EDTA and 0.02% trypsin for approximately 3 min at 37 °C and were used for subsequent bioassays.

Melanogenesis. The effects of compounds on melanogenesis were examined using B16 melanoma 4A5 cells according to the reported protocol.[13] The melanoma cells (8.0×10^3 cells/200 μL/well) were seeded into 48-well multiplates. After 24 h of culture, a test compound and 1 mM theophylline were added and incubated for 72 h. After incubation, the medium was removed, and 105 μL/well of distilled H_2O was added. The cells were homogenized by sonication and lysed with 6 M NaOH (20 μL/well).

An aliquot (100 μL) of the lysate was transferred to a 96-well microplate, and the optical density of each well was measured with a microplate reader (SH-9000, CORONA) at 405 nm (reference: 655 nm). The test compound was dissolved in DMSO, and the final concentration in the medium was 0.1%. The rates of melanin production were corrected on the basis of the viability of melanoma cells.

Inhibition (%) was calculated using the following formula, where *A* and *B* indicate the optical density of vehicle-treated and test compound-treated groups, respectively, and *C* indicates cell viability (%) (vide infra).

$$\text{Inhibition (\%)} = [(A - B)/A]/(C/100) \times 100$$

IC_{50} values were determined graphically on figures including only nontoxic concentrations of compounds.

Viability of Melanoma Cells. Cell viability was assessed according to the protocol in our previous report[13] with slight modification. The melanoma cells (4.0×10^3 cells/100 μL/well) were seeded into 96-well microplates and incubated for 24 h. After 72 h of incubation with 1 mM theophylline and a test compound, 10 μL of 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) {5 mg/mL in [PBS (−)]} solution was added to the medium. After 4 h of incubation, the medium was removed, and 100 μL of isopropanol containing 0.04 M HCl was added to dissolve formazan produced in the cells. The optical density of the formazan was measured at a wavelength of 570 nm (reference: 655 nm). The test compound was dissolved in DMSO, and the final concentration in the medium was 0.1%.

Cell viability (%) was calculated using the following formula, where *A* and *B* indicate the optical density of vehicle-treated and test compound-treated groups, respectively.

$$\text{Cell viability (\%)} = B/A \times 100$$

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

^1H and ^{13}C NMR spectra. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/np500995z.

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Notes

The authors declare no competing financial interest.

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