A concise total synthesis of puberulic acid, potent antimalarial agent

Electronic Supplementary Information (ESI)

Goh Sennari, Tomoyasu Hirose, Masato Iwatsuki, Satoshi Ōmura and Toshiaki Sunazuka

Graduate School of Infection Control Sciences, Kitasato University
Kitasato Institute for Life Sciences, Kitasato University
5-9-1 Shirokane, Minato-ku, Tokyo 108-8641, Japan.

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1. General Methods and Material

Unless otherwise noted, reagents and solvents were purchased at highest commercial quality and used without further purification. Dry acetone, PhMe, DMF, THF, \( \text{CH}_2\text{Cl}_2 \), MeOH, PhH were purchased from Kanto Chemical Co., Inc. Pre-coated silica gel plates with a fluorescent indicator (Merck 60 F254) were used for analytical (0.25 mm) and preparative (0.25 or 0.50 mm) thin layer chromatography. Flash chromatography was carried out with Kanto Chemical silica gel (Kanto Chemical, silica gel 60N, spherical neutral, 0.040–0.050 mm, Cat.-No. 37563–84) or Merck silica gel 230-400 mesh ASTM (60N, 0.040-0.063 mm, Cat.-No. 109385). ODS column chromatography was carried out with Sep-Pak® Plus C18 Short Cartridge (Waters Co. Ltd.) or CHROMATOREX® (Fuji Silysia Chemical Ltd.). \(^1\)H NMR spectra were recorded on JEOL JNM-ECA-500 (500 MHz) and \(^13\)C NMR spectra were recorded on JEOL JNM-ECA-500 (125 MHz). Chemical shifts are expressed in ppm downfield from the internal solvent peaks for CDCl\(_3\) (\(^1\)H; \( \delta = 7.26 \text{ ppm} \), \(^{13}\)C; \( \delta = 77.0 \text{ ppm} \)), CD\(_3\)OD (\(^1\)H; \( \delta = 3.31 \text{ ppm} \), \(^{13}\)C; \( \delta = 49.0 \text{ ppm} \)), CD\(_2\)Cl\(_2\) (\(^1\)H; \( \delta = 5.32 \text{ ppm} \), \(^{13}\)C; \( \delta = 53.84 \text{ ppm} \)), (CD\(_3\))\(_2\)CO (\(^1\)H; \( \delta = 2.05 \text{ ppm} \), \(^{13}\)C; \( \delta = 29.8, 206.3 \text{ ppm} \)) and \( J \) values are given in Hertz. The following abbreviations were used to explain the multiplicities: s = singlet, d = doublet, dd = double doublet, ddd = double double doublet, m = multiplet, br = broad. Infrared spectra for all compounds were measured on a Horiba FT-210 spectrometer. High- and Low-resolution mass spectra were measured on a JEOL JMS-AX505 HA, JEOL JMS-700 MStation and JEOL JMS-T100LP. Optical rotations were measured by using JASCO P-1010 polarimeter.
2. Experimental Procedures and Compounds Characterization

2-1. Champion data of total synthesis of puberulic acid

1,2:3,4-Di-O-isopropylidene-α-D-galactopyranose: S1

![D-(-)-Galactose (10)](image) → ZnCl₂, H₂SO₄, Acetone, r.t. 97% → S1

To a solution of anhydrous ZnCl₂ (28.37 g, 0.21 mol) in acetone (0.28 L) was added H₂SO₄ (0.96 mL, 18.09 mmol) and D-(-)-galactose (25.00 g, 0.14 mol) at room temperature. The mixture was stirred at room temperature for 16 h, and then quenched with sat. aq. NaHCO₃ (300 mL). The resulted suspension was filtrated through a pad of Celite®, then the Celite® pad was washed with acetone. The filtrate was concentrated under reduced pressure to remove excess acetone and extracted with ether (300 mL × 3). The combined organic layers were dried over sodium sulfate, and concentrated under reduced pressure. The residue was purified by silica gel flash column chromatography (hexane/EtOAc = 3/1) to afford S1 (35.03 g, 97%) as a colorless oil:

Rf = 0.38 (hexane/EtOAc = 1/1); ¹H NMR (500 MHz, CDCl₃) δ 5.57 (d, J = 4.6 Hz, 1H), 4.61 (dd, J = 7.7, 2.3 Hz, 1H), 4.34 (dd, J = 4.6, 2.3 Hz, 1H), 4.28 (dd, J = 7.7, 1.7 Hz, 1H), 3.88 (dd, J = 8.0, 6.9, 1.7 Hz, 1H), 3.87 (dd, J = 14.9, 6.9 Hz, 1H), 3.75 (dd, J = 14.9, 8.0 Hz, 1H), 1.54 (s, 3H), 1.46 (s, 3H), 1.34 (s, 6H); identical with the reported data.¹)
1,2,3,4-Di-O-isopropylidene-6-iodo-D-galactopyranose: **11**

To a solution of **S1** (12.00 g, 46.10 mmol) in toluene (0.23 L) was added I$_2$ (8.20 g, 64.62 mmol), Ph$_3$P (18.00 g, 68.63 mmol) and imidazole (9.53 g, 0.14 mol) at room temperature. The mixture was stirred at 70 °C for 15 h. After the reaction was completed, the resulted suspension was filtrated through a pad of Celite®, then the Celite® pad was washed with EtOAc, and the filtrate was concentrated under reduced pressure. The residue was purified by silica gel flash column chromatography (hexane/EtOAc = 15/1) to afford **11** (16.55 g, 97%) as a white solid:

R$_f$ = 0.75 (hexane/EtOAc = 1/1); $^1$H NMR (500 MHz, CDCl$_3$) δ 5.55 (d, $J = 4.6$ Hz, 1H), 4.62 (dd, $J = 7.7$, 2.3 Hz, 1H), 4.41 (dd, $J = 7.7$, 1.7 Hz, 1H), 4.30 (dd, $J = 4.6$, 2.3 Hz, 1H), 3.95 (ddd, $J = 6.9$, 6.9, 1.7 Hz, 1H), 3.32 (dd, $J = 10.3$, 6.9 Hz, 1H), 3.21 (dd, $J = 10.3$, 6.9 Hz, 1H), 1.54 (s, 3H), 1.45 (s, 3H), 1.35 (s, 3H), 1.34 (s, 3H); identical with the reported data.$^1$
2-Chloromethyl-3-(p-methoxybenzyloxy)-prop-1-ene: 12

To a solution of PMBOH (24.88 mL, 0.20 mol) in THF (0.13 L) and DMF (0.04 L) was added NaH (10.40 g, 0.26 mol) at 0 °C. The mixture was refluxed for 4 h, then cooled to room temperature, and added to a solution of metallyl dichloride (25.00 g, 0.20 mol) in THF (0.13 L) dropwise by additional funnel over 1 h. After being stirred at room temperature for 5 min, the resulted mixture was quenched with sat. aq. NH₄Cl (350 mL). The organic layer was separated, and the aqueous layer was extracted with EtOAc (350 mL). The combined organic layers were washed with brine (500 mL), dried over sodium sulfate, and concentrated under reduced pressure. The residue was purified by silica gel flash column chromatography (hexane/EtOAc = 10/1) to afford 12 (29.60 g, 65%) as a pale yellow oil:

Rf = 0.50 (hexane/EtOAc = 3/1); ¹H NMR (500 MHz, CDCl₃) δ 7.27 (d, J = 8.6 Hz, 2H), 6.89 (d, J = 8.6 Hz, 2H), 5.31 (s, 1H), 5.25 (s, 1H), 4.46 (s, 2H), 4.13 (s, 2H), 4.09 (s, 2H), 3.81 (s, 3H); identical with the reported data.²)
To a solution of 11 (4.00 g, 10.81 mmol) in THF (86.48 mL) and H₂O (17.30 mL) was added Zn powder (mesh 75~150 µm; 7.19 g, 0.11 mol) at room temperature, and the mixture was sonicated for 4 h. After consumption of all the starting material, 12 (4.90 g, 21.62 mmol) was added, and then the mixture was stirred vigorously at room temperature for 2 d. The suspension was filtrated by Celite® and the filtrate was extracted with CHCl₃ (100 mL), and washed with brine (100 mL). The residue was purified by silica gel flash column chromatography (hexane/EtOAc = 2/1) to afford (6S)-13 (2.74 g, 67%) and (6R)-13 (1.19 g, 29%), respectively as both colorless oils:

(6S)-13: Rf = 0.42 (hexane/EtOAc = 1/1); ¹H NMR (500 MHz, CDCl₃) δ 7.25 (d, J = 8.6 Hz, 2H), 6.87 (d, 8.6 Hz, 2H), 6.12-6.05 (m, 1H), 5.34 (dd, J = 8.0, 7.5 Hz, 1H), 5.27 (dd, J = 7.5, 1.7 Hz, 1H), 4.46 (d, J = 4.6 Hz, 2H), 3.96 (d, J = 8.0, 1.7 Hz, 1H), 2.71 (d, J = 14.3, 1.7 Hz, 1H), 2.17 (d, J = 14.3, 9.74 Hz, 1H), 1.53 (s, 3H), 1.40 (s, 3H); ¹³C NMR (125 MHz, CDCl₃) δ 159.5, 142.9, 134.8, 129.7, 119.6, 117.3, 114.0, 108.6, 79.5, 76.5, 73.7, 72.3, 72.0, 71.6, 55.4, 55.4, 38.9, 27.0, 25.0; IR (KBr) v max 3462, 2987, 2935, 2838, 1736, 1736, 1612, 1585, 1514, 1464, 1373, 1302, 1248, 1213, 1173, 1039, 893, 847, 820 cm⁻¹; HRMS-ESI (m/z) [M+Na]⁺ calcd for C₂₁H₃₀O₅Na 401.1912, found 401.1931; [α]D²⁷ +53.1 (c 1.0, CHCl₃)

(6R)-13: Rf = 0.28 (hexane/EtOAc = 1/1); ¹H NMR (500 MHz, CDCl₃) δ 7.26 (d, J = 8.6 Hz, 2H), 6.88 (d, J = 8.6 Hz, 2H), 6.03-5.96 (m, 1H), 5.34 (d, J = 17.2 Hz, 1H), 5.27
(d, $J = 10.3$ Hz, 1H), 5.15 (s, 1H), 5.05 (s, 1H), 4.61 (dd, $J = 8.0$, 7.5 Hz, 1H), 4.44 (s, 2H), 4.28 (dd, $J = 6.9$, 4.6 Hz, 1H), 3.96 (d, $J = 7.5$ Hz, 2H), 3.81 (s, 3H), 3.80-3.77 (m, 1H), 3.50 (dd, $J = 4.6$, 2.3 Hz, 1H), 2.40-2.32 (m, 2H), 1.53 (s, 3H), 1.39 (s, 3H); $^{13}$C NMR (125 MHz, CDCl$_3$) δ 159.4, 142.8, 134.2, 130.1, 129.6, 119.9, 116.1, 114.0, 109.1, 79.5, 79.0, 73.3, 72.1, 71.1, 70.8, 55.4, 38.3, 27.4, 25.2; IR (KBr) $\nu_{\text{max}}$ 3369, 3066, 2970, 2904, 1645, 1610, 1512, 1464, 1387, 1302, 1250, 1217, 1165, 1115, 1030, 926, 899, 847, 810, 698, 646 cm$^{-1}$; HRMS-ESI (m/z) [M+Na]$^+$ calcd for C$_{21}$H$_{30}$O$_6$Na 401.1912, found 401.1913; $[\alpha]_D^{26}$ = -49.8 (c 1.0 CHCl$_3$)
(3S,4S,5S,6S)-3,4-O-Isopropylidene-5,6-dihydroxy-1-(p-methoxybenzyloxy)methyl-cyclohept-1-ene: (6S)-14

(3S,4S,5S,6R)-3,4-O-Isopropylidene-5,6-dihydroxy-1-(p-methoxybenzyloxy)methyl-cyclohept-1-ene: (6R)-14

**Synthesis of single diastereomer (6S)-14**

To a solution of (6S)-13 (2.50 g, 6.61 mmol) in CH$_2$Cl$_2$ (0.66 L) was added Grubbs 2nd generation catalyst (0.56 g, 0.66 mmol) at room temperature. The mixture was refluxed for 4 h, and then filtrated through a pad of Celite® to remove reacted catalyst. After concentration of the filtrate under reduced pressure, the residue was purified by silica gel flash column chromatography (hexane/EtOAc = 1/1) to afford (6S)-14 (2.10 g, 91%) as a colorless oil.

R$_f$ = 0.48 (hexane/EtOAc = 1/1); $^1$H NMR (500 MHz, CDCl$_3$) δ 7.25 (d, J = 8.6 Hz, 2H), 6.88 (d, J = 8.6 Hz, 2H), 5.68 (d, J = 1.5 Hz, 1H), 5.00 (br-dd, J = 7.5, 1.5 Hz, 1H), 4.42 (d, J = 1.7 Hz, 2H), 4.32 (dd, J = 10.3, 7.5 Hz, 1H), 4.10 (dd, J = 10.9, 6.9, 4.6 Hz, 1H), 3.89 (d, J = 1.7 Hz, 1H), 3.80 (s, 3H), 3.67 (dd, J = 10.3, 4.6 Hz, 1H), 2.53 (dd, J = 13.5, 6.9 Hz, 1H), 2.08 (br-dd, J = 13.5, 10.9 Hz, 1H), 1.48 (s, 3H), 1.39 (s, 3H); $^{13}$C NMR (125 MHz, CDCl$_3$) δ 159.3, 132.7, 130.2, 129.4, 126.1, 113.9, 108.1, 75.6, 73.8, 73.6, 72.1, 71.3, 66.2, 55.4, 32.2, 27.4, 24.6; IR (KBr) ν$_{max}$ 3473, 3078, 2987, 2935, 2839, 1612, 1514, 1464, 1381, 1302, 1250, 1213, 1174, 1036, 926, 876, 820, 675 cm$^{-1}$; HRMS-ESI (m/z) [M+Na]$^+$ calcd for C$_{19}$H$_{26}$O$_6$Na 373.1614, found 373.1632; [α]$_D^{26}$ +44.1 (c 1.0 CHCl$_3$)
Synthesis of single diastereomer (6R)-14

According to the procedure for preparation of (6S)-14, ring-closing metathesis of (6R)-13 (2.80 g, 7.40 mmol) afforded (6R)-14 (2.54 g, 98%) as a white solid:

\[ \text{Rf} = 0.30 \text{ (hexane/EtOAc = 1/1); } ^1\text{H NMR (500 MHz, CDCl}_3\text{) } \delta 7.26 (d, J = 8.6 \text{ Hz}, 2H), 6.88 (d, J = 8.6 \text{ Hz}, 2H), 5.71 (s, 1H), 4.91 (d, J = 7.5 \text{ Hz}, 1H), 4.49 (d, J = 7.5 \text{ Hz}, 2H), 3.99 (dd, J = 7.5, 10.3 \text{ Hz}, 1H), 3.86 (d, J = 4.6 \text{ Hz}, 2H), 3.80 (s, 3H), 3.63 (ddd, J = 6.9, 4.6, 2.3 Hz, 1H), 3.48 (dd, J = 10.3, 6.9 Hz, 1H), 2.38 (dd, J = 14.9, 4.6 Hz, 1H), 2.24 (dd, J = 14.9, 2.3 1H), 1.48 (s, 3H), 1.37 (s, 3H); ^1\text{C NMR (125 MHz, CDCl}_3\text{) } \delta 159.5, 132.7, 129.7, 129.5, 127.8, 114.0, 108.3, 76.7, 76.4, 75.0, 73.7, 72.9, 71.3, 55.4, 33.5, 27.3, 24.3; \text{IR (KBr) } v_{\text{max}} \text{ 3460, 2987, 2935, 2839, 1736, 1612, 1514, 1464, 1373, 1302, 1248, 1211, 1174, 1043, 897, 874, 820, 723 cm}^{-1}; \text{ HRMS-ESI (m/z) [M+Na]^+ } \text{ calcd for C}_{11}\text{H}_{18}\text{O}_5\text{Na 253.1052, found 253.1051; } [\alpha]_{D}^{25} \text{ } -43.0 \text{ (c 1.0 CHCl}_3\text{); mp 115} \text{ } ^{\circ}\text{C}

Synthesis using diastereomixture of 13

According to the procedure for preparation of (6S)-14, ring-closing metathesis of two diastereomixtures (d.r. = 1:1) of 13 (0.12 g, 0.31 mmol) afforded 14 (0.11 g, 97%) as colorless oil.
(3S,4S,5S,6S)-3,4,5,6-Tetrahydroxy-1-(p-methoxybenzyl)oxymethyl-cyclohept-1-ene: (6S)-15

To a solution of (6S)-14 (0.51 g, 1.46 mmol) in MeOH (48.67 mL) was added TsOH•H₂O (13.8 mg, 72.54 µmol) at room temperature. The mixture was stirred at room temperature for 22 h, then the resulted suspension was concentrated under reduced pressure. The residue was purified by silica gel flash column chromatography (CHCl₃/MeOH = 4/1) to afford (6S)-15 (0.45 g, 99%) as a white amorphous:

Rf = 0.26 (CHCl₃/MeOH = 10/1); ¹H NMR (500 MHz, CD₃OD) δ 7.28 (d, J = 8.6 Hz, 2H), 6.89 (d, J = 8.6 Hz, 2H), 5.59 (s, 1H), 4.72 (br-d, J = 2.9 Hz, 1H), 4.44 (d, J = 11.5 Hz, 1H), 4.40 (d, J = 11.5 Hz, 1H), 4.35 (br-dd, J = 5.2, 2.9 Hz, 1H), 3.90 (s, 2H), 3.86 (d, J = 5.2 Hz, 1H), 3.82 (d, J = 10.9 Hz, 1H), 3.78 (s, 3H), 2.76 (dd, J = 14.3, 10.9 Hz, 1H), 2.00 (d, J = 14.3 Hz, 1H); ¹³C NMR (125 MHz, CD₃OD) δ 160.6, 135.1, 132.7, 131.4, 130.6, 114.5, 77.5, 75.9, 75.5, 72.3, 69.3, 67.6, 55.5, 31.9; IR (KBr) v max 3373, 2908, 2839, 1614, 1587, 1514, 1464, 1362, 1302, 1250, 1174, 1113, 1059, 1036, 941, 852, 818, 759, 687 cm⁻¹; HRMS-FAB (m/z) [M+Na]⁺ calcd for C₁₆H₂₂O₆Na 333.1314, found 333.1326

※ Optical rotations could not be measured due to low solubility of the pure amorphous.
(3S,4S,5S,6S)-3,4-O-Isopropylidene-5,6-dihydroxy-1-hydroxymethyl-cyclohept-1-ene: (6S)-18
(3S,4S,5S,6R)-3,4-O-Isopropylidene-5,6-dihydroxy-1-hydroxymethyl-cyclohept-1-ene: (6R)-18

**Synthesis of single diastereomer (6S)-18**

To a solution of (6S)-14 (0.40 g, 1.14 mmol) in CH$_2$Cl$_2$ (10.26 mL) and pH 7 buffer (1.14 mL) was added DDQ (0.39 g, 1.71 mmol) at 0 °C. The mixture was stirred at 0 °C for 5 h. After the reaction was completed, to the resulted suspension was added Celite® and H$_2$O (30 mL), then filtrated through a pad of Celite®, and the Celite® pad was washed with H$_2$O (300 mL). The filtrate was extracted with CHCl$_3$ (300 mL), and the aqueous phase was concentrated under reduced pressure. The residue was purified by silica gel flash column chromatography (CHCl$_3$/MeOH = 10/1) to afford (6S)-18 (0.22 g, 83%) as a colorless oil:

R$_f$ = 0.36 (CHCl$_3$/MeOH = 10/1); $^1$H NMR (500 MHz, CD$_3$OD) δ 5.56 (br-s, 1H), 4.97 (br-d, J = 7.5 Hz, 1H), 4.28 (dd, J = 9.2, 7.5 Hz, 1H), 4.01 (ddd, J = 8.6, 6.3, 3.4 Hz, 1H), 3.94 (s, 2H), 3.66 (dd, J = 9.2, 3.4, 1H), 2.43 (dd, J = 14.9, 6.3 Hz, 1H), 2.15 (dd, J = 14.9, 8.6 Hz, 1H), 1.44 (s, 3H), 1.37 (s, 3H); $^{13}$C NMR (125 MHz, CD$_3$OD) δ 136.5, 126.1, 108.8, 77.7, 76.1, 73.8, 69.0, 66.8, 34.6, 27.5, 24.6; IR (Diamond prism) $\nu_{\text{max}}$ 3379, 2989, 2931, 2893, 2877, 2854, 1454, 1373, 1261, 1211, 1161, 1088, 1038, 887, 864, 845, 791, 752, 667, 633 cm$^{-1}$; HRMS-FAB (m/z) [M+Na]$^+$ calcd for C$_{11}$H$_{15}$O$_5$Na 253.1052, found 253.1050; $[\alpha]_D^{24}$ +61.6 (c 0.1 MeOH)
Synthesis of single diastereomer \((6R)-18\)

According to the procedure for preparation of \((6S)-18\), deprotection of \((6R)-14\) (0.10 g, 0.29 mmol) afforded \((6R)-18\) (54.7 mg, 82%) as a white solid:

\[ R_f = 0.33 \ (\text{CHCl}_3/\text{MeOH} = 10/1); \ \text{\textsuperscript{1}H NMR (500 MHz, CD}_3\text{OD}) \ \delta 5.64 \ (\text{d, } J = 1.7 \text{ Hz}, 1\text{H}), 4.97 \ (\text{br-d, } J = 7.5 \text{ Hz}, 1\text{H}), 4.04 \ (\text{dd, } J = 10.3, 7.5 \text{ Hz}, 1\text{H}), 4.03 \ (\text{d, } J = 13.8 \text{ Hz}, 1\text{H}), 3.95 \ (\text{d, } J = 13.8 \text{ Hz}, 1\text{H}), 3.62 \ (\text{ddd, } J = 6.9, 4.6, 2.9 \text{ Hz}, 1\text{H}), 3.38 \ (\text{dd, } J = 10.3, 6.9 \text{ Hz}, 1\text{H}), 2.37 \ (\text{dd, } J = 14.9, 4.6 \text{ Hz}, 1\text{H}), 2.10 \ (\text{br-d, } J = 14.9 \text{ Hz}, 1\text{H}), 1.46 \ (\text{s, } 3\text{H}), 1.37 \ (\text{s, } 3\text{H}); \ \text{\textsuperscript{13}C NMR (125 MHz, CD}_3\text{OD}) \ \delta 137.0, 124.3, 108.7, 78.1, 76.8, 76.3, 72.8, 66.5, 33.7, 27.3, 24.2; \ \text{IR (Diamond prism) } \nu_{\text{max}} \text{ 3448, 3367, 2993, 2931, 2854, 1450, 1373, 1331, 1250, 1219, 1176, 1153, 1095, 1072, 1041, 1007, 910, 879, 845, 822, 802, 694, 648 \text{ cm}^{-1}; \ \text{HRMS-FAB (m/z) [M+Na]}^{+} \text{ calcd for C}_{11}\text{H}_{16}\text{O}_{3}\text{Na, found; } [\alpha]_{D}^{23} -54.5 \ (c \ 0.1 \text{ MeOH}); \ \text{mp 110 } ^{\circ}\text{C} \]

Synthesis using diastereomixture of 14

According to the procedure for preparation of \((6S)-18\), deprotection of two diastereomixtures of 14 (0.10 g, 0.28 mmol) afforded 18 (60.3 mg, 94%) as colorless oil.
2,3-\textit{O}-Isopropylidene-7-methoxy-5-formyl-tropone: \textbf{21}

3,4-\textit{O}-Isopropylidene-2-methoxy-6-formyl-tropone: \textbf{S2}

To a solution of two diastereomixtures of \textbf{18} (21.7 mg, 94.24 µmol) in CH$_2$Cl$_2$ (0.94 mL) was added DMSO (0.10 mL, 1.41 mmol), Et$_3$N (0.39 mL, 2.83 mmol), and SO$_3$•Pyridine (0.15 g, 0.94 mmol) at 0 °C. The mixture was stirred at room temperature for 20 h, monitored by LC-UV analysis, then quenched with 1M aq. HCl (10 mL), and extracted with CHCl$_3$ (10 mL × 3). The combined organic layers were dried over sodium sulfate, and concentrated under reduced pressure to yield crude product as a dark brown amorphous. This crude product was used in subsequent reaction without further purification.

To a stirred solution of crude product in DMF (0.94 mL) in sealed tube was added K$_2$CO$_3$ (0.13 g, 0.94 mmol) and MeI (58.52 µL, 0.94 mmol) at room temperature. The mixture was stirred at 50 °C for 2 h, then quenched with 1M aq. HCl (10 mL), and extracted with CHCl$_3$ (10 mL × 3). The organic layer was dried over sodium sulfate, and concentrated under reduced pressure. The residue was purified by preparative TLC (EtOAc) to afford \textbf{21} (3.2 mg, 14% over 2 steps) as a yellow solid and \textbf{S2} (3.1 mg, 14% over 2 steps) as a yellow solid:

\textbf{21}: R$_f$ = 0.18 (EtOAc); $^1$H NMR (500 MHz, CD$_2$Cl$_2$) δ 9.75 (s, 1H), 7.32 (d, $J$ = 1.2 Hz, 1H), 7.29 (br-d, 1H), 4.01 (s, 3H), 1.78 (s, 6H); $^{13}$C NMR (125 MHz, CD$_3$OD) δ 191.3, 167.1, 164.7, 151.1, 149.7, 133.8, 122.1, 119.4, 105.3, 56.9, 26.2; IR (Diamond prism) $\nu_{max}$ 1685, 1597, 1577, 1469, 1442, 1369, 1288, 1219, 1142, 1084, 1049, 1049, 1007, 980, 937, 868, 802, 779, 729 cm$^{-1}$; HRMS-El (m/z) [M+Na]$^+$ calcd for C$_{12}$H$_{12}$O$_5$ 236.0685, found 236.0688; mp 232 °C

\textbf{S2}: R$_f$ = 0.57 (EtOAc); $^1$H NMR (500 MHz, CD$_2$Cl$_2$) δ 9.65 (s, 1H), 7.17 (d, $J$ = 1.2 Hz, 1H), 6.83 (d, $J$ = 1.2 Hz, 1H), 3.95 (s, 3H), 1.75 (s, 6H); $^{13}$C NMR (125 MHz, CD$_3$OD)
δ 193.8, 179.6, 155.8, 152.0, 148.8, 140.5, 138.8, 118.8, 99.8, 60.0, 26.3; IR (Diamond prism) νmax 1705, 1635, 1566, 1442, 1385, 1350, 1254, 1211, 1161, 1119, 1041, 964, 941, 856, 798, 779, 706, 648 cm⁻¹; HRMS-EI (m/z) [M+Na]⁺ calcd for C₁₂H₁₂O₅ 236.0685, found 236.0688; mp 87 °C

**NOE observation of 21 and S2:**

※ NOE correlation between methoxy group at C2 position and 7-H of S2 was not observed.
2,3-\(\text{O-Isopropylidene-7-methoxy-5-methoxycarbonyl-tropone: 24}\)
3,4-\(\text{O-Isopropylidene-2-methoxy-6-methoxycarbonyl-tropone: 25}\)

According to the procedure for preparation of 21, Parikh-Doering oxidation of two diastereomixtures of 18 (42.2 mg, 0.18 mmol) afforded crude product as a dark brown amorphous. This crude material was used in subsequent reaction without further purification.

To a stirred solution of crude product and 2-methyl-2-butene (0.39 mL, 3.66 mmol) in THF (1.83 mL) and \(t\)-BuOH (1.83 mL) was added the mixture of NaClO\(_2\) (33.1 mg, 0.37 mmol) and Na\(\text{H}_2\)PO\(_4\) (0.11 g, 0.73 mmol) in H\(_2\)O (1.83 mL) dropwise by Pasteur pipette. The mixture was stirred at room temperature for 5 min, monitored by UC-UV analysis, then quenched with 1M aq. HCl (20 mL), and extracted with CHCl\(_3\) (30 mL \(\times\) 3). The combined organic layers were dried over sodium sulfate, and concentrated under reduced pressure to yield crude product as a dark brown amorphous. This crude material was used in subsequent reaction without further purification.

To a stirred solution of crude product in PhH (1.65 mL) and MeOH (0.18 mL) was added TMSCHN\(_2\) (0.6M in hexane; 0.64 mL, 0.38 mmol) at room temperature. The mixture was stirred at room temperature for 3 h, and then concentrated under reduced pressure at 20 °C. The residue was purified by preparative TLC (EtOAc) to afford 24 (10.0 mg, 21% over 3 steps) as a yellow solid and 25 (9.9 mg, 21% over 3 steps) as a yellow solid:

\(24: \text{RF} = 0.08 (\text{hexane/EtOAc = 1/1}); \text{H NMR} (500 MHz, CD\(_3\)OD) \delta 7.84 (s, 1H), 7.73 (s, 1H), 4.03 (s, 3H), 3.96 (s, 3H), 1.79 (s, 6H); \text{C NMR} (125 MHz, CD\(_3\)OD) \delta 167.4, 167.3, 165.1, 152.5, 152.0, 130.9, 120.5, 118.2, 111.3, 56.8, 53.9, 25.8; \text{IR} (Diamond
prism) $v_{\text{max}}$ 1712, 1577, 1500, 1423, 1381, 1342, 1296, 1214, 1111, 1076, 1003, 876, 768, 652 cm$^{-1}$; HRMS-ESI ($m/z$) [M+Na]$^+$ calcd for C$_{13}$H$_{14}$O$_6$Na 289.0688, found 289.0689; mp 187 °C

25: $R_f$ = 0.36 (hexane/EtOAc = 1/1); $^1$H NMR (500 MHz, CD$_3$OD) $\delta$ 7.62 (d, $J=1.7$ Hz, 1H), 7.16 (d, $J=1.7$ Hz, 1H), 3.94 (s, 3H), 3.92 (s, 3H), 1.79 (s, 6H); $^{13}$C NMR (125 MHz, CD$_3$OD) $\delta$ 181.0, 167.7, 156.7, 153.5, 149.9, 136.4, 134.3, 120.5, 106.0, 60.5, 54.0, 26.0; IR (Diamond prism) $v_{\text{max}}$ 2954, 1724, 1562, 1427, 1385, 1350, 1250, 1207, 1157, 1065, 1018, 941, 860, 779, 737, 675 cm$^{-1}$; HRMS-ESI ($m/z$) [M+Na]$^+$ calcd for C$_{13}$H$_{14}$O$_6$Na 289.0688, found 289.0689; mp 113 °C

**NOE observation of 21 and S2:**

※ NOE correlation between methoxy group at C2 position and 7-H of 25 was not observed.
Puberulic acid: 1

According to the procedure for preparations of 24 and 25, Parikh-Doering oxidation of two diastereomixtures of 18 (20.5 mg, 89.03 µmol) and subsequent Pinnick oxidation afforded crude product as a dark brown amorphous. This crude material was used in subsequent reaction without further purification.

The solution of crude product in 33% HBr/AcOH (0.89 mL) was refluxed for 16 h and monitored by LC-UV analysis. After the reaction was completed, the resulted mixture was concentrated under reduced pressure. The residue was purified by Sep-pak® Plus C18 Short Cartridge to afford puberulic acid (11.5 mg, 65% over 3 steps) as a yellow solid:

\[ \text{H NMR (500 MHz, (CD}_3\text{)}_2\text{CO) } \delta 7.94 \text{ (s, 2H); (500 MHz, CD}_3\text{OD) } \delta 7.90 \text{ (s, 2H); } \text{C} \text{ NMR (125 MHz, (CD}_3\text{)}_2\text{CO) } \delta 167.5, 159.5, 155.5, 128.3, 119.3; (125 MHz, CD}_3\text{OD) } \delta 168.6, 159.7, 156.0, 128.5, 119.2; IR (Diamond prism) } v_{max} 3502, 3259, 3143, 3051, 2808, 2511, 1697, 1593, 1535, 1389, 1342, 1284, 1180, 1053, 1014, 903, 775, 733, 687 \text{ cm}^{-1}; \text{ HRMS-EI (m/z) [M]}^+ \text{ calcd for C}_8\text{H}_6\text{O}_6 198.0164, \text{ found 198.0154; mp 258 °C} \]

※ CD$_3$OD solvent of NMR analysis is better due to low-solubility in (CD$_3$)$_2$CO.
2,3-\textit{O}-Isopropylidene-5-formyl-tropolone: \textbf{22}

2,3-\textit{O}-Isopropylidene-5-carboxy-tropolone: \textbf{23}

The synthetic intermediates of puberulic acid, which are troponoids \textbf{22} and \textbf{23}, were purified by Sep-pak® Plus C18 Short Cartridge for the measurement of spectra data. The experimental procedures are according to preparation of \textbf{24} and \textbf{25}:

\textbf{22}: a yellow solid; \textsuperscript{1}H NMR (500 MHz, (CD\textsubscript{3})\textsubscript{2}CO) \(\delta\) 9.89 (s, 1H), 7.54 (d, \(J = 1.2\) Hz, 1H), 7.49 (d, \(J = 1.2\) Hz, 1H), 1.81 (s, 6H); \textsuperscript{13}C NMR (125 MHz, (CD\textsubscript{3})\textsubscript{2}CO) \(\delta\) 192.9, 166.6, 163.1, 154.0, 149.4, 137.4, 120.6, 116.7, 112.7, 26.2; IR (Diamond prism) \(\nu_{\text{max}}\) 3209, 2931, 2823, 2723, 1701, 1581, 1550, 1500, 1392, 1350, 1315, 1184, 1119, 1038, 960, 837, 771, 687 cm\textsuperscript{-1}; HRMS-FAB (\textit{m}/\textit{z}) [M+H]\textsuperscript{+} calcd for \(\text{C}_{11}\text{H}_{11}\text{O}_{5}\) 223.0606, found 223.0606; mp 196 \degree C

\textbf{23}: a yellow solid; \textsuperscript{1}H NMR (500 MHz, CD\textsubscript{3}OD) \(\delta\) 7.89 (d, \(J = 1.7\) Hz, 1H), 7.74 (br-d, \(J = 1.7\) Hz, 1H), 1.80 (s, 6H); \textsuperscript{13}C NMR (125 MHz, CD\textsubscript{3}OD) \(\delta\) 168.3, 166.8, 163.4, 154.6, 150.3, 134.5, 120.7, 116.2, 115.4, 26.0; IR (Diamond prism) \(\nu_{\text{max}}\) 3155, 2850, 2592, 1697, 1628, 1581, 1504, 1462, 1408, 1365, 1315, 1211, 1103, 1026, 872, 764, 702, 629 cm\textsuperscript{-1}; HRMS-ESI (\textit{m}/\textit{z}) [M+H]\textsuperscript{+} calcd for \(\text{C}_{11}\text{H}_{11}\text{O}_{6}\) 239.0556, found 239.0547; mp 261 \degree C

S18
2-2. For large-scale synthesis of puberulic acid

In gram-scale synthesis of puberulic acid, the deprotection of PMB group with DDQ was inefficient and irreproducibility, probably because the concentration of huge amount of the aqueous phase with residual hydroquinone caused the decomposition of the product. So Birch reduction was applied in this step, although the yield was inferior to the deprotection using DDQ.

Large-scale synthesis of (6S)-18

![Chemical structure of (6S)-14 and (6S)-18]

To a solution of Li (0.15 g, 22.26 mmol) in liq. NH₃ (34.25 mL) was added the mixture of (6S)-14 (1.20 g, 3.42 mmol) in THF (34.25 mL) and t-BuOH (0.71 mL) dropwise under Ar atmosphere. The mixture was stirred at −78 °C for 5 min, then quenched with isoprene (3 mL), and warmed to room temperature to remove liq. NH₃. To the resulted suspension was added MeOH (20 mL) and silica gel (100 cc), and the mixture was concentrated under reduced pressure. The dried silica gel was eluted with MeOH, and the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (CHCl₃/MeOH = 20/1) to afford (6S)-18 (0.73 g, 92%) as a colorless oil.

Purification of large-scale puberulic acid (1)

According to the procedure for preparations of puberulic acid (1), Parikh-Doering oxidation of (6S)-18 (1.03 g, 4.47 mmol), subsequent Pinnick oxidation and deprotection of the acetonide group afforded crude product as a dark brown amorphous. This crude material was dissolved in 1% MeOH/0.1% TFA aq. (1.5 L) and applied on reverse-phase column chromatography (50 φ × 130 mm, CHROMATOREX®). After washing 5% and 10% MeOH/0.1% TFA aq. (400 mL each), I was eluted with 15, 20, 25 and 30% MeOH/0.1% TFA aq. (400 mL each), followed by concentration under reduced pressure to afford I (0.40 g, 45%) as a yellow solid.
2-3. Comparison of the NMR data of synthetic and natural puberulic acid

![Puberulic acid diagram]

<table>
<thead>
<tr>
<th>Position</th>
<th>Synthetic puberulic acid&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Reported one&lt;sup&gt;b,3)&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;sup&gt;13&lt;/sup&gt;C NMR</td>
<td>&lt;sup&gt;1&lt;/sup&gt;H NMR</td>
</tr>
<tr>
<td>Position</td>
<td>δ&lt;sub&gt;C&lt;/sub&gt; (ppm)</td>
<td>δ&lt;sub&gt;H&lt;/sub&gt; (ppm), [mult., Int.]</td>
</tr>
<tr>
<td>1</td>
<td>159.5</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>155.5</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>119.3</td>
<td>7.94 (s, 2H)</td>
</tr>
<tr>
<td>4</td>
<td>128.3</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>167.5</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>exp. <sup>13</sup>C NMR: 125 MHz, <sup>1</sup>H NMR: 500 MHz in (CD<sub>3</sub>)<sub>2</sub>CO

<sup>b</sup>exp. <sup>13</sup>C NMR: 75 MHz, <sup>1</sup>H NMR: 300 MHz in (CD<sub>3</sub>)<sub>2</sub>CO
2-4. Determination of stereochemistry at C6 position

(3S,4S,5S,6S)-3,4:5,6-O-Isopropylidene-1-(p-methoxybenzyl)oxy)methyl-cyclohept-1-ene: S3

To a solution of (6S)-14 (20.0 mg, 57.08 µmol) in 2,2-dimethoxypropane (0.57 mL) was added PPTS (0.14 mg, 5.71 µmol) at room temperature. The mixture was stirred at room temperature for 24 h, then quenched with sat. aq. NaHCO₃ (3 mL), and extracted with EtOAc (5 mL). The organic layer was dried over sodium sulfate, and concentrated under reduced pressure. The residue was purified by preparative TLC (hexane/EtOAc = 1/2) to afford S3 (15.8 mg, 71%) as a colorless oil:

\[ \text{Rf} = 0.55 \text{ (hexane/EtOAc = 1/2); } \]
\[ ^1\text{H NMR (500 MHz, CDCl}_3\text{)} \delta 7.26 (d, J = 8.6 Hz, 2H), 6.88 (d, J = 8.6 Hz, 2H), 5.76 (s, 1H), 4.93 (d, J = 7.5 Hz, 1H), 4.45 (s, 2H), 4.35 (dd, J = 12.6, 6.9, 4.6 Hz, 1H), 4.28 (dd, J = 10.3, 7.5 Hz, 1H), 3.99 (dd, J = 10.3, 6.9 Hz, 1H), 3.92 (s, 2H), 3.81 (s, 3H), 2.48 (dd, J = 13.2, 4.6 Hz, 1H), 2.23 (dd, J = 13.2, 12.6 Hz, 1H), 1.54 (s, 3H), 1.53 (s, 3H), 1.39 (s, 3H), 1.34 (s, 3H); \]
\[ ^{13}\text{C NMR (125 MHz, CDCl}_3\text{)} \delta 159.4, 133.1, 130.1, 129.4, 125.8, 114.0, 109.3, 107.8, 78.3, 74.5, 73.9, 73.5, 72.2, 55.4, 29.1, 28.3, 27.8, 25.3, 24.7; \]
\[ \text{IR (KBr) } \nu_{\text{max}} 2989, 2937, 2844, 1738, 1612, 1583, 1514, 1462, 1381, 1302, 1248, 1215, 1161, 1065, 1038, 970, 906, 852, 820, 756 \text{ cm}^{-1}; \]
\[ \text{HRMS-ESI (m/z) [M+Na]^+ calecd for } C_{22}H_{30}O_6Na 413.1916, \text{ found 413.1915; } [\alpha]_D^{25} +18.5 (c 1.0 CHCl}_3\text{)} \]

NOE observations of S3:

S21
(3S,4S,5S,6R)-3,4:5,6-O-Isopropylidene-1-(p-methoxybenzoyloxy)methyl-cyclohept-1-ene: S4

According to the procedure for preparation of S3, protection of (6R)-14 (16.2 mg, 46.23 µmol) afforded S4 (15.2 mg, 84%) as a colorless oil:

Rf = 0.58 (hexane/EtOAc = 1/2); $^1$H NMR (500 MHz, CDCl$_3$) δ 7.25 (d, $J$ = 8.6 Hz, 2H), 6.88 (d, $J$ = 8.6 Hz, 2H), 5.67 (s, 1H), 4.83 (br-d, $J$ = 6.9 Hz, 1H), 4.41 (s, 2H), 4.29 (dd, $J$ = 6.9, 6.9 Hz, 1H), 3.95 (ddd, $J$ = 9.8, 9.2, 5.2 Hz, 1H), 3.92 (dd, $J$ = 9.2, 6.9 Hz, 1H), 3.87 (s, 2H), 3.81 (s, 3H), 2.84 (dd, $J$ = 17.8, 5.2 Hz, 1H), 2.52 (dd, $J$ = 17.8, 9.8 Hz, 1H), 1.49 (s, 3H), 1.45 (s, 3H), 1.44 (s, 3H), 1.39 (s, 3H); $^{13}$C NMR (125 MHz, CDCl$_3$) δ 159.4, 132.2, 130.1, 129.5, 126.2, 113.9, 110.8, 109.4, 81.6, 80.3, 75.9, 73.6, 73.5, 71.9, 55.4, 35.3, 27.7, 27.3, 27.2, 25.3; IR (KBr) $v_{\text{max}}$ 2987, 2935, 2900, 2841, 1728, 1614, 1514, 1462, 1371, 1248, 1217, 1173, 1105, 1051, 899, 852, 814, 758 cm$^{-1}$; HRMS-ESI ($m/z$) [M+Na]$^+$ calced for C$_{22}$H$_{30}$O$_6$Na 413.1916, found 413.1918; [$\alpha$]$_D^{26}$ = 14.4 (c 1.0 CHCl$_3$)

ROESY observations of S4:

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S22
3. Spectra Charts

3-1. $^1$H NMR and $^{13}$C NMR charts
(6S)-18
(in CD$_3$OD)
(in CD$_3$OD)
(in CDCl3)
(in CDCl₃)
3-2. LC-UV analytical conditions and chromatograms

LC-UV analysis was carried out with LC-UV Agilent 1100 Series (Agilent Technology Inc.) under following conditions.

Column: Symmetry C18 (Waters Co. Ltd., 2.1 φ × 150 mm)
Mobile phase: 5-100\% CH$_3$CN/0.05\% H$_3$PO$_4$ (linear gradient over 20 min)
Flow rate: 0.2 mL/min
Detection: photodiode array (190-600 nm)
Column temperature: 40 °C

The reactions to form the tropolone skeleton were monitored with LC-UV analysis. Its chromatograms and UV spectra are shown below (Retention Time: R. T.).
• Synthesis of aldehyde 22 (Parikh-Doering oxidation)

\[
\begin{array}{c}
\text{SO}_2\cdot\text{Pyridine} \\
\text{DMSO, Et}_3\text{N} \\
\text{CH}_2\text{Cl}_2 \\
0 \degree \text{C to r.t.}
\end{array}
\]

LC-UV chart (210 nm)

UV absorption (mAU) vs. Retention time (min)

Product 22 (R. T. = 10.55 min)

UV spectrum (R. T. = 10.55 min)
• Synthesis of carboxylic acid 23 (Pinnick oxidation)

\[
\begin{align*}
\text{NaClO}_2, \quad \text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}, \\
\text{2-Methyl-2-buten}, \quad \text{THF/}t\text{-BuOH/H}_2\text{O, r.t.}
\end{align*}
\]

UV spectrum (R. T. = 8.85 min)

LC-UV chart (210 nm) over 2 steps

UV absorption (mAU)

Retention time (min)

Product 23 (R. T. = 8.85 min)

Reagent

UV spectrum (R. T. = 8.85 min)
• Synthesis of Puberulic acid

\[
\begin{align*}
\text{HBr/AcOH} & \\
\text{sealed tube} & \\
120 \degree C & \\
\end{align*}
\]

LC-UV chart (210 nm) over 3 steps

UV spectrum (R. T. = 6.13 min)
4. References

