Supplementary material

Synthesis and study of the pancreatic α-amylase inhibitory activity of methyl acarviosin and its derivatives

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**Experimental procedures**

**General methods.**
All commercially available chemicals were purchased from Wako Pure Chem. Ind. Ltd., unless otherwise noted, and used without further purification. Structures of the synthetic compounds were determined by NMR and Mass spectrometry. Bruker AMX500 or Jeol JNM-EX 270 was used to obtain NMR spectrum and either tetramethylsilane (TMS) or residual solvent peak was used as an internal standard ($^1$H NMR: TMS 0.00 ppm (for CDCl$_3$), CD$_3$OD 3.30 ppm, D$_2$O 4.75 ppm; $^{13}$C NMR: CDCl$_3$ 77.0 ppm, CD$_3$OD 49.0 ppm). Jeol JMS SX-102A (FAB-MS) or Jeol JMS-T100GCV (FD-MS) or Thermo Scientific Exactive (ESI-MS) was used to obtain mass spectrum. Combination of Aquity UPLC system (Waters Co.) and LCT-premier (Waters Co.) were used for UPLC-Tof-MS analysis. Absorbance was measured by Synergy™ MX (Bio-tech Instruments Inc.,) microplate reader.

**Determination of pancreatic amylase inhibitory activity**
The α-amylase inhibitory activity was determined by using the method described by Ali *et al.* with a modification.$^1$ Porcine pancreatic amylase (PPA, Sigma-Aldrich Co.) was dissolved in sodium phosphate buffer (20 mM, pH 6.9) containing 6.7 mM of NaCl to give a concentration of 1 unit/mL solution. Soluble starch (10 mg/mL) in the same buffer was used as a substrate solution. A coloring reagent (DNS) was prepared by mixing 4.8 M aq. 3,5-dinitrosalicylic acid (20 mL) and a solution of (+)-sodium potassium tartrate tetrahydrate (12 g) in 2 M NaOH (8 mL). A sample solution (100 μL) in 50% dimethyl sulfoxide (DMSO) and the PPA solution (150 μL) were mixed in a micro tube (1.5 mL). The tube was pre-incubated at 37 °C for 15 min. The starch solution (250 μL) was then added and the mixture was incubated at 37 °C for 15 min. To terminate the reaction, the tube was dipped in boiling water for 1 min. After cooling, the reaction mixture was directly passed through a small ODS (Cosmosil 75C18-OPN, Nacalai Tesque Co., Kyoto, Japan) column (3 mL) to remove any sample constituents that may interfere with the following color reaction. The reaction mixture (100 μL) was then mixed with coloring reagent (50 μL) and heated in boiling water for 15 min. The mixture was cooled on ice and then diluted with water (450 μL). The obtained solution (200 μL) was transferred into 96-well micro plate, and the optical density was determined at a wavelength of 540 nm. The control experiment was performed using 50% aq. DMSO in place of the sample solution. Blank experiments for sample and control were performed using the sodium phosphate buffer in place of the enzyme solution and each blank value was subtracted from the sample and the control values, respectively. The inhibitory activity (%) was calculated as \[\{1-\frac{A_{540 \text{ (sample)}}}{A_{540 \text{ (control)}}}\} \times 100\]. Each experiment is tested in duplicate and repeated at least twice.
**UPLC-Tof-MS analysis of the enzyme reaction mixture**

The enzyme reaction was performed according to the above method and the mixture before passing through the small ODS column was used for the analysis. UPLC-Tof-MS analysis was achieved using Waters Acquity UPLC / LCT Premier (Waters Co.) system with ESI as an ion source. Inertsustain C18 column (2 μm, φ2.1 mm×100 mm, GL Science Co.) was employed for the separation with the following elution condition.

**Mobile phase:**
- 0 to 10 min: 5% aq. methanol with 0.1% formic acid
- 10 to 20 min: gradient elution from 5% aq. methanol up to 95% aq. methanol, with 0.1% formic acid
- Flow rate: 0.20 mL/min.

Mass spectrum was measured by positive mode between \( m/z \) 100-1500.

Supplementary Figure 1. Total ion chromatogram (TIC) and selected ion chromatogram (SIC) of the UPLC-Tof-MS analysis of enzyme reaction mixture with methyl α-acarviosin (1) as the inhibitor. A: SIC, \( m/z \) 680.27 corresponding to [methyl α-acarviosin + maltose+H]+; B: SIC, \( m/z \) 498.22 corresponding to [methyl α-acarviosin + glucose+H]+; C: SIC, \( m/z \) 336.16 corresponding to [methyl α-acarviosin+H]+; D: TIC.
Supplementary Figure 2. TIC and SIC of the UPLC-Tof-MS analysis of enzyme reaction mixture with compound 6 as the inhibitor. A: SIC, \(m/z\) 894.37 corresponding to \([6 + \text{maltose} + \text{H}]^+\); B: SIC, \(m/z\) 731.33 corresponding to \([6 + \text{glucose} + \text{H}]^+\); C: SIC, \(m/z\) 570.27 corresponding to \([6 + \text{H}]^+\); D: TIC.
Supplementary Figure 3. TIC and SIC of the UPLC-Tof-MS analysis of enzyme reaction mixture with compound 7 as the inhibitor. A: SIC, m/z 922.42 corresponding to [7 + maltose + H]^+; B: SIC, m/z 760.36 corresponding to [7 + glucose + H]^+; C: SIC, m/z 597.29 corresponding to [7 + H]^+; D: TIC.
Supplementary Figure 4. TIC and SIC of the UPLC-Tof-MS analysis of enzyme reaction mixture with compound 8 as the inhibitor. A: SIC, m/z 964.46 corresponding to [8 + maltose + H]^+; B: SIC, m/z 802.42 corresponding to [8 + glucose + H]^+; C: SIC, m/z 640.32 corresponding to [8 + H]^+; D: TIC.
Supplementary Figure 5. TIC and SIC of the UPLC-Tof-MS analysis of enzyme reaction mixture with compound 9 as the inhibitor. A: SIC, $m/z$ 674.28 corresponding to [9 + maltose + H]$^+$; B: SIC, $m/z$ 512.23 corresponding to [9 + glucose + H]$^+$; C: SIC, $m/z$ 350.17 corresponding to [9 + H]$^+$; D: TIC.
Acarbose hydrate (7.03 g)(TCI Co., A2485) was dissolved in 10% acetyl chloride/methanol (100 mL) and stirred for 14 hrs at 70°C. The reaction mixture was cooled and evaporated to dryness. The residue was purified by silica-gel column chromatography (stepwise elution: ethyl acetate/methanol/water = 60/8/1 → 40/8/1 → 5/4/1) to obtain methyl acarviosin as α and β mixture (2.59 g, 7.72 mmol). Anomer mixture of acarviosin was then dissolved in a 1:5 mixture of DMF and 2,2-dimethoxypropane, and catalytic amount of camphorsulfonic acid (CSA) was added. The mixture was stirred for 2 hrs at 60°C. The reaction mixture was cooled, diluted with sat. aq. sodium hydrogen carbonate and extracted by ethyl acetate. The organic layer was washed with brine, dried over sodium sulfate and evaporated. The residue was separated by silica-gel column chromatography (stepwise elution: hexane/ethyl acetate = 5/1 → 4/1 → 2/1) to obtain isopropylidene protected α-anomer (640 mg, 1.40 mmol) and β-anomer (570 mg, 1.25 mmol) separately. Part of the α-anomer (7.1 mg, 0.0156 mmol) was then dissolved in a 1:1 mixture of 1 M hydrogen chloride solution and THF (2.0 mL) and stirred for an hour. The reaction mixture was evaporated and the residue was purified by silica-gel column chromatography (ethyl acetate/methanol/water = 40/8/1) to obtain methyl α-acarviosin (I, 4.7 mg, 0.0140 mmol, 89%).
Isopropylidene protected methyl acarviosin

\(
\alpha\)-anomer\(^{82}\):
\(\text{\textsuperscript{1}H-NMR (270 MHz, CDCl}_3, \text{rt}): \delta 1.33 \text{ (3H, d, } J = 6.2 \text{ Hz), 1.41 (s, 3H), 1.44 (s, 6H), 1.46 (s, 3H), 1.47 (s, 3H), 1.57 (s, 3H), 2.78 (1H, t, } J = 9.7 \text{ Hz), 3.37-3.46 (4H, m), 3.50 (1H, dd, } J = 3.2, 9.7 \text{ Hz), 3.59 (1H, dd, } J = 4.8, 9.9 \text{ Hz), 3.84 (1H, t, } J = 9.7 \text{ Hz), 4.04 (1H, t, } J = 4.8 \text{ Hz), 4.14 (1H, dd, } J = 8.5, 9.9 \text{ Hz), 4.19 (1H, d, } J = 13.5 \text{ Hz), 4.48 (1H, d, } J = 13.5 \text{ Hz), 4.53 (1H, d, } J = 8.5 \text{ Hz), 4.98 (1H, d, } J = 3.2 \text{ Hz), 5.68 (1H, d, } J = 4.8 \text{ Hz ppm); ESI-MS (positive): } m/z = 456 ([M+Na]^+)\)

\(\beta\)-anomer:\(\text{\textsuperscript{1}H-NMR (270 MHz, CDCl}_3, \text{rt}): \delta 1.38 \text{ (1H, d, } J = 6.3 \text{ Hz), 1.42 (6H, s), 1.44 (3H, s), 1.47 (6H, s), 1.57 (3H, s), 2.82 (1H, t, } J = 9.0 \text{ Hz), 3.17-3.45 (3H, m), 3.55 (3H, s), 3.60 (1H, dd, } J = 4.6, 9.8 \text{ Hz), 4.03 (1H, t, } J = 4.6 \text{ Hz), 4.09 (1H, dd, } J = 8.2, 9.8 \text{ Hz), 4.20 (1H, d, } J = 13.8 \text{ Hz), 4.44-4.55 (3H, m), 5.66 (1H, d, } J = 4.7 \text{ Hz) ppm; } ^{13}\text{C-NMR (67.5 MHz, CDCl}_3, \text{rt}): \delta 18.11, 20.03, 26.44, 26.68, 26.72, 27.06, 27.91, 50.93, 56.44, 60.07, 62.96, 71.70, 74.40, 75.20, 77.09, 77.63, 82.17, 99.03, 102.24, 111.05, 111.41, 121.73, 133.78 ppm; HR ESI MS (positive): [M+H]^+ \text{ found } m/z 456.2592, \text{ C}_{23}\text{H}_{38}\text{NO}_8^+ \text{ requires } m/z 456.2597; \text{ IR (neat) } \nu: 755, 843, 1107, 1236, 1373, 2934, 2986 \text{ cm}^{-1}; [\alpha]_D^{21} +101.8^\circ (c 1.00, \text{ CHCl}_3)\)

Methyl \(\alpha\)-acarviosin (1)\(^{83}\)
\(\text{\textsuperscript{1}H-NMR (270 MHz, D}_2\text{O, rt): 1.31 (3H, d, } J = 6.3 \text{ Hz), 2.43 (1H, t, } J = 9.7 \text{ Hz), 3.36 (3H, s), 3.48-3.58 (3H, m), 3.59-3.76 (3H, m), 4.00 (1H, d, } J = 6.6 \text{ Hz), 4.07 (1H, d, } J = 13.8 \text{ Hz), 4.19 (1H, d, } J = 13.8 \text{ Hz), 4.71 (1H, d, } J = 3.0 \text{ Hz), 5.86 (1H, d, } J = 5.0 \text{ Hz ppm; ESI-MS (positive): } m/z = 336 ([M+H]^+)\)
Synthesis of selectively protected methyl α-acarviosin (4)

Methyl 4,6-O-benzylidene α-acarviosin (2)

\[
\begin{align*}
\text{1} & \text{) 80\% AcOH aq, 1,4-dioxane} \\
\text{2) } & \text{α,α-dimethoxytoluene, CSA} \\
\text{3) 40\% AcOH aq, 1,4-dioxane} \\
\end{align*}
\]

Isopropylidene protected methyl α-acarviosin (640 mg, 1.41 mmol) was dissolved in 1,4-dioxane (5.0 mL) and 80\%aq. acetic acid (30.0 mL). After stirring for 6 hrs at 50°C, the reaction mixture was evaporated. The residue was dissolved in DMF (4.0 mL) and α,α-dimethoxytoluene (20 mL), CSA (32.8 mg, 0.14 mmol) was added. After stirring for 2 hrs at 80°C, the reaction mixture was cooled, triethylamine (TEA) was added and evaporated. The residue was dissolved in 1,4-dioxane (15.0 mL) and 40\%aq. acetic acid (15.0 mL). After stirring for 14 hrs, the reaction mixture was evaporated and the residue was purified by silica-gel column chromatography (ethyl acetate/methanol = 20/1 then ethyl acetate/methanol/water = 20/8/1) to obtain 2 (248 mg, 0.586 mmol, 42\%) as a white solid.

\[\text{1H-NMR (270 MHz, CD3OD, rt): 1.30 (3H, d, } J = 6.3 \text{ Hz), 2.34 (1H, t, } J = 9.6 \text{ Hz), 3.35 (3H, s), 3.38-3.57 (5H, m), 3.88 (1H, dd, } J = 7.5, 9.7 \text{ Hz), 4.26 (1H, d, } J = 7.5 \text{ Hz), 4.42 (1H, d, } J = 12.9 \text{ Hz), 4.50 (1H, d, } J = 12.9 \text{ Hz), 4.58 (1H, d, } J = 3.6 \text{ Hz), 5.69 (1H, s), 5.76 (1H, brd, } J = 3.7 \text{ Hz), 7.29-7.36 (3H, m), 7.44-7.51 (2H, m) ppm; 13C-NMR (67.5 MHz, CD3OD, rt): 18.68, 55.49, 58.61, 68.09, 69.86, 70.89, 72.13, 73.71, 74.77, 75.36, 81.18, 101.4, 102.31, 123.76, 127.60, 129.19, 129.98, 132.76, 139.83 ppm; HR FD MS (positive): [M+H]+ found m/z 424.19809, C21H30NO8+ requires m/z 424.19714; IR (neat) v: 700, 750, 1061, 1110, 1367, 1455, 2904, 3367 cm⁻¹; [α]D^{23} +155.4° (c 1.00, CH3OH)\]

Methyl 2,3,2’,3’-tetra-O-p-methoxybenzyl-4,6-O-benzylidene α-acarviosin (3)

\[
\begin{align*}
\text{2) NaH} \\
\text{DMF} \\
\text{56\%} \\
\end{align*}
\]

Compound 2 (248 mg, 0.586 mmol) was dissolved in anhydrous DMF (6.00 mL) and sodium hydride (144 mg, 6.00 mmol) was added at 0°C under argon atmosphere. After stirring for 30 min at 0°C, 4-methoxybenzyl chloride (0.80 mL, 0.587 mmol) was added and further stirred for 20 hrs at rt. The reaction mixture was further stirred at 70°C for 3 hrs and then cooled to rt. Water was added to quench the reaction and then the mixture was extracted by ethyl acetate. Organic layer was washed with brine, dried over sodium sulfate and evaporated. The residue was purified by silica-gel column

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chromatography (hexane/ethyl acetate = 4/1→2/1) to obtain 3 (295 mg, 0.327 mmol, 56%) as a colorless oil.

1H-NMR (500 MHz, CDCl3, rt): 1.24 (3H, d, J = 6.3 Hz), 2.44 (1H, t, J = 9.6 Hz), 3.35 (3H, s), 3.47-3.52 (3H, m), 3.65 (1H, t, J = 9.2 Hz), 3.74 (3H, s), 3.77 (3H, s), 3.78 (3H, s), 3.79 (3H, s), 3.98 (1H, J = 4.3 Hz), 4.30 (1H, dd, J = 6.9, 10.0 Hz), 4.35-4.44 (2H, m), 4.50 (1H, d, J = 3.4 Hz), 4.54 (1H, d, J = 11.7 Hz), 4.56 (1H, d, J = 11.5 Hz), 4.58 (1H, d, J = 10.6 Hz), 4.65 (1H, d, J = 11.7 Hz), 4.69 (1H, d, J = 11.5 Hz), 4.73 (1H, d, J = 10.9 Hz), 4.77 (1H, d, J = 10.9 Hz), 4.95 (1H, d, J = 10.6 Hz), 5.63 (1H, s), 5.67 (1H, d, J = 4.3 Hz), 6.74-6.87 (8H, m), 7.10-7.47 (13H, m) ppm; 13C-NMR (125 MHz, CDCl3, rt): 18.82, 52.97, 54.96, 55.15, 55.20, 60.25, 68.46, 70.26, 72.55, 72.67, 74.51, 74.87, 78.22, 78.60, 80.90, 81.05, 83.43, 97.79, 100.79, 113.59, 113.68, 113.77, 122.97, 126.12, 126.65, 128.09, 128.68, 128.92, 129.16, 129.45, 129.50, 129.69, 130.26, 130.53, 131.12, 131.25, 132.15, 138.21, 158.92, 159.00, 159.06, 159.32 ppm; HR FD MS (positive): [M]+ found m/z 903.42043, C53H61NO12+ requires m/z 903.41937; IR (neat) υ: 821, 1075, 1250, 1514, 1613, 2835 cm⁻¹; [α]D^21 + 13.3° (c 1.00, CHCl₃)

Methyl 2,3,2’,3’-tetra-O-p-methoxybenzyl-6-O-isopropylsilyl α-acarviosin (4)

![Chemical Structure](image)

Compound 3 (262 mg, 0.290 mmol) was suspended in 80% aq. acetic acid (5.00 mL) and 1,4-dioxane was added until the compound dissolved. The reaction mixture was stirred for overnight and then further stirred for 24 hrs at 50°C. Sat. aq. sodium hydrogen carbonate was added to the reaction mixture and extracted by ethyl acetate. The organic layer was washed with brine, dried over sodium sulfate and evaporated. To the residue was dissolved in dichloromethane (3.00 mL) and triisopropylsilyl chloride (0.100 mL, 0.472 mmol), imidazole (38.4 mg, 0.564 mmol) was added at 0°C. The reaction mixture was stirred for overnight under argon atmosphere. Water was added to the reaction mixture and extracted by ethyl acetate. The organic layer was washed with brine, dried over sodium sulfate and evaporated. The residue was purified by silica-gel column chromatography (hexane/ethyl acetate = 4/1→2/1) to obtain 4 (183 mg, 0.188 mmol, 65%) as a colorless oil.

1H-NMR (500 MHz, CDCl3, rt): 1.03-1.15 (21H, m), 1.22 (3H, d, J = 6.2 Hz), 2.44 (1H, t, J = 9.7 Hz), 3.36 (3H, s), 3.49 (1H, dd, J = 3.5, 9.5 Hz), 3.52 (1H, dd, J = 6.3, 9.7 Hz), 3.62-3.68 (2H, m), 3.74 (3H, s), 3.76 (3H, s), 3.78 (3H, s), 3.79 (3H, s), 3.93 (1H, br t, J = 3.8 Hz), 4.00 (1H, dd, J = 4.3, 6.9 Hz), 4.06 (1H, d, J = 4.3 Hz), 4.27 (1H, d, J = 13.9 Hz), 4.31 (1H, d, J = 13.9 Hz), 4.47-4.62 (7H, m), 4.65 (1H, d, J = 11.7 Hz), 4.91 (1H, d, J = 10.6 Hz), 5.81 (1H, d, J = 3.8 Hz), 6.73-6.89 (8H, m), 7.12-7.26 (8H, m) ppm; 13C-NMR (125 MHz, CDCl3, rt): 11.89, 17.99, 18.91, 52.79, 54.98, 55.13, 55.15, 55.17, 61.30, 64.58, 68.04, 69.82, 72.38, 72.57, 72.63, 74.79, 78.46, 78.61, 80.72, 82.75, 97.92, 113.60, 113.72, 123.25, 128.94, 129.21, 129.32, 129.65, 130.09, 130.29, 130.78, 131.09,
138.99, 158.84, 159.08, 159.17, 159.28 ppm; HR FD MS (positive): [M]$^+$ found m/z 971.52132, $\text{C}_{55}\text{H}_{77}\text{NO}_{12}\text{Si}^+$ requires m/z 971.52150; IR (neat) $\nu$: 821, 1173, 1302, 1464, 1613, 2865, 2865, 2939, 3514 cm$^{-1}$; $\lbrack \alpha \rbrack_D^{21} +26.3^\circ$ (c 1.00, CHCl$_3$)
Synthesis of iodoalkyl glucose (5a-c)

2,3,4,6-tetra-O-benzyl-1-O-(4-hydroxybutyl)-α-D-glucopyranose (10a)

2,3,4,6-tetra-O-benzyl-D-glucopyranose (1.23 g, 2.28 mmol) was dissolved in dichloromethane (20.0 mL) and triphenylphosphine (2.09 g, 7.97 mmol), carbon tetrabromide (2.35 g, 7.08 mmol) was added and stirred for 14 hrs at rt. To this reaction mixture, 1,4-butandiol (448 mg, 4.98 mmol), 1,1,3,3-tetramethylurea (TMU, 1.64 mL, 13.7 mmol), tetraethylammonium bromide (TEAB, 575 mg, 2.74 mmol) dissolved in THF (20.0 mL) was added dropwise and stirred for 2 days at rt. The reaction mixture was diluted by 1 M hydrogen chloride and extracted by ethyl acetate. The organic layer was washed with brine, dried over sodium sulfate and evaporated. The residue was purified by silica-gel column chromatography (hexane/ethyl acetate = 4/1→3/1→1/1) to obtain 10a (773 mg, 1.26 mmol, 55%) as a colorless oil.

1H-NMR (270 MHz, CDCl3, rt): 1.55-1.78 (4H, m), 3.38-3.49 (1H, m), 3.55 (1H, dd, J= 3.5, 9.6 Hz), 3.59-3.80 (7H, m), 3.97 (1H, t, J= 9.3 Hz), 4.47 (2H, d, J= 11.9 Hz), 4.59 (1H, d, J=12.2 Hz), 4.64 (1H, d, J=12.9 Hz), 4.74 (1H, d, J= 3.6 Hz), 4.76-4.86 (3H, m), 4.98 (1H, d, J=10.9 Hz), 7.11-7.17 (2H, m), 7.24-7.37 (18H, m) ppm; 13C-NMR (67.5 MHz, CDCl3, rt): 25.94, 29.84, 62.35, 67.96, 68.50, 70.21, 73.17, 73.41, 75.02, 75.60, 76.53, 77.00, 77.48, 77.68, 79.89, 82.03, 97.02, 127.58, 127.73, 127.91, 128.11, 128.39, 128.47, 137.97, 138.27, 138.89 ppm; HR FD MS (positive): [M+Na]+ found m/z 635.2995, C38H44O7Na+ requires m/z 635.2985; IR (neat) ν: 680, 737, 1071, 1361, 1454, 2868, 2919, 3482 cm⁻¹; [α]D25 +26.7° (c 1.00, CHCl3)
2,3,4,6-tetra-O-benzyl-1-O-(4-(acetyloxy)butyl)-\(\alpha\)-D-glucopyranose (11a)

Compound 10a (773 mg, 1.26 mmol) was dissolved in anhydrous dichloromethane (12.0 mL) and TEA (0.260 mL, 1.87 mmol), acetic anhydride (0.140 mL, 1.48 mmol) was added. After stirring for overnight, TEA (0.260 mL, 1.87 mmol), acetic anhydride (0.140 mL, 1.48 mmol), and catalytic amount of 4-dimethylaminopyridine (DMAP) was added. The reaction mixture was stirred for an hour and then diluted by chloroform. The solution was washed by sat. aq. sodium hydrogen carbonate and then by brine. The organic layer was dried over sodium sulfate and evaporated. The residue was purified by silica-gel column chromatography (hexane/ethyl acetate = 10/1→5/1) to obtain 11a (717 mg, 1.10 mmol, 87%) as a colorless oil.

\begin{align*}
\text{1H-NMR} & \quad (500 \text{ MHz, CDCl}_3, \text{ rt}): 1.65-1.74 \ (4 \text{H, m}), 2.03 \ (3 \text{H, s}), 3.39-3.46 \ (1 \text{H, m}), 3.56 \ (1 \text{H, dd, } J = 3.7, 9.7 \text{ Hz}), 3.61-3.96 \ (3 \text{H, m}), 3.72 \ (1 \text{H, dd, } J = 3.7, 10.4 \text{ Hz}), 4.60 \ (1 \text{H, d, } J = 12.3 \text{ Hz}), 4.82 \ (1 \text{H, d, } J = 10.7 \text{ Hz}), \text{ ppm; 13C-NMR} \quad (67.5 \text{ MHz, CDCl}_3, \text{ rt}): 20.83, 25.37, 25.86, 64.13, 67.54, 68.47, 70.19, 73.17, 73.44, 75.08, 75.62, 77.72, 80.11, 82.04, 97.04, 127.60, 127.74, 127.77, 127.91, 127.96, 128.02, 128.44, 128.49, 138.02, 138.31, 138.41, 138.98, 171.27 \text{ ppm; HR FD MS (positive): } [M]^+ \text{ found } m/z \ 654.31932, \ C_{40}H_{46}O_{8}^+ \text{ requires } m/z \ 654.31927; \ \text{IR (neat)}: \ \nu: 698, 738, 1071, 1244, 1363, 1455, 1736, 2869, 2918 \text{ cm}^{-1}; \ \left[\alpha\right]_D^{25} +28.7^\circ \ (c \ 1.00, \text{CHCl}_3) \end{align*}

2,3;4,6-di-O-isopropylidene-1-O-(4-(acetyloxy)butyl)-\(\alpha\)-D-glucopyranose (12a)

Compound 11a (717 mg, 1.10 mmol) was dissolved in methanol (8.00 mL) and THF (2.00 mL). The solution was adjusted to pH3 by addition of 1 M aq. hydrogen chloride. Palladium hydroxide on carbon (53.6 mg) was added to the solution and stirred for 90 min. under hydrogen atmosphere. The reaction mixture was passed through Celite® pad and evaporated. The residue was dissolved in 2,2-dimethoxypropane (10.0 mL) and DMF (1.00 mL), CSA (14.1 mg, 0.0607 mmol) was added and stirred for overnight at 70ºC. TEA was added to the reaction mixture and the evaporated. The residue was purified by silica-gel column chromatography (hexane/ethyl acetate = 8/1→2/1) to obtain 12a (287 mg, 0.767 mmol, 70%) as a colorless oil.

\begin{align*}
\text{1H-NMR} & \quad (500 \text{ MHz, CDCl}_3, \text{ rt}): 1.65-1.74 \ (4 \text{H, m}), 2.03 \ (3 \text{H, s}), 3.39-3.46 \ (1 \text{H, m}), 3.56 \ (1 \text{H, dd, } J = 3.7, 9.7 \text{ Hz}), 3.61-3.96 \ (3 \text{H, m}), 3.72 \ (1 \text{H, dd, } J = 3.7, 10.4 \text{ Hz}), 4.60 \ (1 \text{H, d, } J = 12.3 \text{ Hz}), 4.82 \ (1 \text{H, d, } J = 10.7 \text{ Hz}), \text{ ppm; 13C-NMR} \quad (67.5 \text{ MHz, CDCl}_3, \text{ rt}): 20.83, 25.37, 25.86, 64.13, 67.54, 68.47, 70.19, 73.17, 73.44, 75.08, 75.62, 77.72, 80.11, 82.04, 97.04, 127.60, 127.74, 127.77, 127.91, 127.96, 128.02, 128.44, 128.49, 138.02, 138.31, 138.41, 138.98, 171.27 \text{ ppm; HR FD MS (positive): } [M]^+ \text{ found } m/z \ 654.31932, \ C_{40}H_{46}O_{8}^+ \text{ requires } m/z \ 654.31927; \ \text{IR (neat)}: \ \nu: 698, 738, 1071, 1244, 1363, 1455, 1736, 2869, 2918 \text{ cm}^{-1}; \ \left[\alpha\right]_D^{25} +28.7^\circ \ (c \ 1.00, \text{CHCl}_3) \end{align*}
1H-NMR (270 MHz, CDCl3, rt): 1.43-1.48 (9H, m), 1.55 (3H, s), 1.69-1.77 (4H, m), 2.06 (3H, s), 3.49-3.60 (3H, m), 3.72-3.81 (1H, m), 3.83-3.94 (3H, m), 4.04 (1H, t, J = 9.3 Hz), 4.11 (2H, t, J = 6.3 Hz), 5.13 (1H, d, J = 3.1 Hz) ppm; 13C-NMR (67.5 MHz, CDCl3, rt): 18.99, 20.78, 25.31, 25.91, 26.16, 26.67, 28.88, 62.29, 64.03, 65.06, 68.04, 73.71, 73.93, 76.82, 97.87, 99.65, 111.39, 171.18 ppm; HR FD MS (positive): [M]+ found m/z 374.19538, C18H30O8+ requires m/z 374.19407; IR (neat) \( \nu \): 854, 966, 1092, 1239, 1372, 1740, 2938, 2989 cm\(^{-1}\); \([\alpha]D^2_{23} + 69.6^\circ (c \ 1.00, \ CHCl_3)\)

2,3;4,6-di-O-isopropylidene-1-O-(4-iodobutyl)-\(\alpha\)-D-glucopyranose (5a)

Compound 12a (287 mg, 0.767 mmol) was dissolved in methanol (4.00 mL) and THF (1.00 mL). Potassium carbonate (89.7 mg, 0.650 mmol) was added to the solution and stirred for 6.5 hrs at rt. The reaction mixture was diluted with water and extracted by ethyl acetate. The organic layer was washed with brine, dried over sodium sulfate and evaporated. The residue was dissolved in dichloromethane (8.00 mL) and triphenylphosphine (239 mg, 0.911 mmol), imidazole (79.6 mg, 1.17 mmol), N-iodosuccinimide (NIS, 215 mg, 0.956 mmol) was added. After stirring for overnight, the reaction mixture was diluted with chloroform, washed with sat. aq. sodium hydrogen carbonate and brine. The organic layer was dried over sodium sulfate and evaporated. The residue was purified by silica-gel column chromatography (hexane/ethyl acetate = 8/1→4/1) to obtain 5a (293 mg, 0.663 mmol, 86%) as a colorless oil.

1H-NMR (270 MHz, CDCl3, rt): 1.45 (6H, s), 1.47 (3H, s), 1.55 (3H, s), 1.68-1.83 (2H, m), 1.88-2.01 (2H, m), 3.25 (2H, t, J = 6.8 Hz), 3.48-3.59 (3H, m), 3.73-3.94 (4H, m), 4.03 (1H, t, J = 9.4 Hz), 5.12 (1H, d, J = 3.1 Hz) ppm; 13C-NMR (67.5 MHz, CDCl3, rt): 6.41, 18.99, 26.22, 26.68, 28.88, 30.10, 30.18, 62.28, 65.09, 67.54, 73.70, 73.88, 76.76, 97.90, 99.66, 111.43 ppm; HR FD MS (positive): [M]+ found m/z 442.08667, C16H27IO6+ requires m/z 442.08523; IR (neat) \( \nu \): 839, 956, 1092, 1203, 1373, 1456, 2933, 2988 cm\(^{-1}\); \([\alpha]D^2_{23} + 60.3^\circ (c \ 1.00, \ CHCl_3)\)

2,3,4,6-tetra-O-benzyl-1-O-(6-hydroxyhexyl)-\(\alpha\)-D-glucopyranose (10b)

2,3,4,6-tetra-O-benzyl-D-glucopyranose (912.3 mg, 1.69 mmol) was dissolved in dichloromethane (15.0 mL) and triphenylphosphine (1.43 g, 5.45 mmol), carbon tetrabromide (1.63 g, 4.91 mmol) was
added and stirred for 3 hrs at rt. To this reaction mixture, 1,4-hexanediol (438 mg, 3.72 mmol), TMU (1.20 mL, 10.0 mmol), TEAB (437 mg, 2.08 mmol) dissolved in THF (15.0 mL) was added dropwise and stirred over night at rt. The reaction mixture was diluted by 1 M hydrogen chloride and extracted by ethyl acetate. The organic layer was washed with brine, dried over sodium sulfate and evaporated. The residue was purified by silica-gel column chromatography (hexane/ethyl acetate = 5/1→2/1) to obtain 10b (507 mg, 0.792 mmol, 47%) as a colorless oil.

$$\text{1H-NMR (500 MHz, CDCl}_3, \text{rt): 1.34-1.44 (4H, m), 1.51-1.58 (2H, m), 1.60-1.67 (2H, m), 3.38-3.46 (1H, m), 3.55 (1H, dd, J = 3.4, 9.3 Hz), 3.57-3.67 (5H, m), 3.71 (1H, dd, J = 3.7 10.5 Hz), 3.75-3.80 (1H, m), 3.98 (1H, t, J = 9.3 Hz), 4.47 (2H, d, J = 11.7 Hz), 4.60 (1H, d, J = 12.1 Hz), 4.64 (1H, d, J =12.2 Hz), 4.75 (1H, d, J = 3.7 Hz), 4.77 (1H, d, J = 12.1 Hz), 4.81 (1H, d, J = 10.9 Hz), 4.83 (1H, d, J =10.7 Hz), 4.99 (1H, d, J = 10.9 Hz), 7.12-7.15 (2H, m), 7.25-7.38 (18H, m) ppm; 13C-NMR (125 MHz, CDCl}_3, \text{rt): 25.46, 25.97, 29.30, 32.61, 62.78, 68.07, 68.67, 70.17, 73.07, 73.45, 75.03, 75.59, 77.86, 80.18, 82.09, 96.91, 127.47, 127.60, 127.63, 127.76, 127.83, 127.87, 127.90, 127.94, 128.31, 128.36, 138.01, 138.29, 138.37, 138.94 ppm; HR ESI MS (positive): [M+Na] + found m/z 663.3286, C_{40}H_{48}O_{7}Na^+ requires m/z 663.3298; IR (neat) \nu: 687, 736, 1069, 2862, 2930, 3446 cm^{-1}; [\alpha]_D^{24} + 22.6^\circ (c 1.00, \text{CHCl}_3 )$$

$2,3,4,6$-tetra-$O$-benzyl-$1-O$-(6-(acetyloxy)hexyl)-$\alpha$-$D$-glucopyranose (11b)

$$\text{Compound 10b (267mg, 0.392 mmol) was dissolved in anhydrous dichloromethane (4.0 mL) and TEA (0.10 mL, 0.719 mmol), acetic anhydride (0.050 mL, 0.529 mmol) was added. After stirring for overnight, the reaction mixture was diluted by chloroform, washed by sat. aq. sodium hydrogen carbonate and then by brine. The organic layer was dried over sodium sulfate and evaporated. The residue was purified by silica-gel column chromatography (hexane/ethyl acetate = 10/1→5/1) to obtain 11b (202 mg, 0.296 mmol, 76%) as a colorless oil.}

$$\text{1H-NMR (500 MHz, CDCl}_3, \text{rt): 1.33-1.43 (4H, m), 1.57-1.67 (4H, m), 2.03 (3H, s), 3.38-3.45 (1H, m), 3.55 (1H, dd, J = 3.7, 9.7 Hz), 3.59-3.67 (3H, m), 3.72 (1H, dd, J = 3.7,10.5 Hz), 3.75-3.79 (1H, m), 3.98 (1H, t, J = 9.3 Hz), 4.04 (2H, t, J = 6.7 Hz), 4.47 (2H, d, J = 12.0 Hz), 4.60 (1H, d, J = 12.1 Hz), 4.64 (1H, d, J = 12.0 Hz), 4.75 (1H, d, J = 3.7 Hz), 4.78 (1H, d, J = 12.0 Hz), 4.82 (1H, d, J =11.0 Hz), 4.83 (1H, d, J = 10.7 Hz), 4.99 (1H, d, J =10.7 Hz), 7.12-7.15 (2H, m), 7.25-7.38 (18H, m) ppm; 13C-NMR (125 MHz, CDCl}_3, \text{rt): 20.95, 25.72, 25.81, 28.47, 29.25, 64.44, 67.99, 68.50, 70.09, 73.08, 73.41, 75.04, 75.60, 77.72, 80.06, 82.06, 96.89, 127.48, 127.61, 127.63, 127.76, 127.83, 127.84, 127.89, 127.94, 128.29, 128.31, 128.34, 137.90, 138.18, 138.29, 138.86, 171.11 ppm; HR ESI MS (positive): [M+Na] ^+ found m/z 705.3412, C_{42}H_{50}O_{7}Na^+ requires m/z 705.3403; IR (neat) \nu: 698, 738, 1071, 1239, 1363, 1455, 1736, 2863, 2931 cm^{-1}; [\alpha]_D^{25} +33.3^\circ (c 1.00, \text{CHCl}_3 )$$
5-3-22. 2,3;4,6-di-O-isopropylidene-1-O-(6-(acetyloxy)hexyl)-α-D-glucopyranose (12b) の合成

Compound 11b (186 mg, 0.253 mmol) was dissolved in methanol (4.00 mL) and THF (1.00 mL). The solution was adjusted to pH3 by addition of 1 M aq. hydrogen chloride. Palladium hydroxide on carbon (53.6 mg) was added to the solution and stirred for 90 min under hydrogen atmosphere. The reaction mixture was passed through Celite® pad and evaporated. The residue was dissolved in 2,2-dimethoxypropane (4.0 mL), DMF (1.00 mL), and CSA (6.0 mg, 0.026 mmol) was added and stirred for overnight at 60°C. After cooling to rt, sat. aq. sodium hydrogen carbonate was added and extracted by ethyl acetate. The organic layer was washed with brine, dried over sodium sulfate and evaporated. The residue was purified by silica-gel column chromatography (hexane/ethyl acetate = 6/1→4/1) to obtain 12b (61.5 mg, 0.174 mmol, 69%) as a colorless oil.

1H-NMR (270 MHz, CDCl3, rt): 1.34-1.48 (13H, m), 1.55 (3H, s), 1.58-1.70 (4H, m), 2.05 (3H, s), 3.46-3.60 (3H, m), 3.66-3.77 (1H, m), 3.82-3.93 (3H, m), 4.00-4.08 (1H, m), 4.06 (2H, t, J = 6.6 Hz), 5.12 (1H, d, J = 3.1 Hz) ppm; 13C-NMR (67.5 MHz, CDCl3, rt): 19.04, 20.86, 25.61, 25.65, 26.22, 26.72, 28.43, 28.93, 29.22, 62.38, 64.42, 65.01, 68.51, 73.79, 74.01, 76.92, 97.90, 99.70, 111.41, 171.34 ppm; HR ESI MS (positive): [M+Na]+ found m/z 425.2140, C20H34O8Na+ requires m/z 425.2151; IR (neat) υ: 840, 1091, 1235, 1372, 1739, 2937 cm⁻¹; [α]D²³ + 95.9° (c 0.720, CHCl₃)

2,3;4,6-di-O-isopropylidene-1-O-(6-iodohexyl)-α-D-glucopyranose (5b) の合成

Compound 12b (61.5 mg, 0.174 mmol) was dissolved in methanol (2.00 mL) and THF (1.00 mL). Potassium carbonate (20.5 mg, 0.149 mmol) was added to the solution and stirred for overnight at rt. The reaction mixture was diluted with water and extracted by ethyl acetate. The organic layer was washed with brine, dried over sodium sulfate and evaporated. The residue was dissolved in dichloromethane (2.00 mL) and triphenylphosphine (54.7 mg, 0.209 mmol), imidazole (18.7 mg, 0.275 mmol), NIS (63.2 mg, 0.281 mmol) was added. After stirring for overnight, the reaction mixture was diluted with chloroform, washed with sat. aq. sodium hydrogen carbonate and brine. The organic layer was dried over sodium sulfate and evaporated. The residue was purified by silica-gel column chromatography (hexane/ethyl acetate = 10/1→5/1) to obtain 5b (56.3 mg, 0.119
mmol, 69%) as a colorless oil.

^1^H-NMR (500 MHz, CDCl₃, rt): 1.37-1.49 (13H, m), 1.55 (3H, s), 1.61-1.69 (2H, m), 1.81-1.88 (2H, m), 3.20 (2H, t, J = 6.9 Hz), 3.49-3.59 (3H, m), 3.70-3.76 (1H, m), 3.83 (1H, t, J = 10.6 Hz), 3.89-3.91 (2H, m), 4.03 (1H, t, J = 9.3 Hz), 5.12 (1H, d, J = 3.1 Hz) ppm; ^1^C-NMR (125 MHz, CDCl₃, rt): 6.87, 19.14, 24.99, 26.35, 26.80, 29.03, 29.19, 30.16, 33.31, 62.39, 65.00, 68.42, 73.76, 73.97, 76.87, 97.82, 99.64, 111.33 ppm; HR ESI MS (positive): [M+Na]^+ found m/z 493.1057, C₁₈H₃₁O₆INa⁺ requires m/z 493.1063; IR (neat) γ: 852, 1092, 1204, 1372, 2873, 2933, 2988 cm⁻¹; [α]D²₃ +56.3° (c 1.00, CHCl₃)

2,3,4,6-tetra-O-benzyl-1-O-(9-(acetyloxy)nonyl)-α-D-glucopyranose (11c)

\[ \text{CBBr₄, Ph₃P, CH₂Cl₂ then TMU, TEAB, 1,4-nonanediol, THF} \]

\[ \text{Ac₂O, TEA, CH₂Cl₂} \]

2,3,4,6-tetra-O-benzyl-D-glucopyranose (1.98 g, 3.67 mmol) was dissolved in dichloromethane (30.0 mL) and triphenylphosphine (3.34 g, 12.7 mmol), carbon tetrabromide (3.58 g, 10.8 mmol) was added and stirred for 3 hrs at rt. To this reaction mixture, 1,4-nonanediol (1.42 g, 8.86 mmol), TMU (2.90 mL, 24.2 mmol), TEAB (1.04 g, 4.95 mmol) dissolved in THF (30.0 mL) was added dropwise and stirred for 24 hrs at rt. The reaction mixture was diluted by 1 M aq. hydrogen chloride and extracted by ethyl acetate. The organic layer was washed with brine, dried over sodium sulfate and evaporated. The residue was partly purified by silica-gel column chromatography (hexane/ethyl acetate = 4/1→2/1) to obtain crude 10c. The crude 10c was dissolved in anhydrous dichloromethane (15.0 mL) and TEA (2.20 mL, 15.8 mmol), acetic anhydride (1.20 mL, 12.7 mmol), catalytic amount of DMAP was added. After stirring for 4.5 hrs, the reaction mixture was diluted by chloroform, washed by sat. aq. sodium hydrogen carbonate and then by brine. The organic layer was dried over sodium sulfate and evaporated. The residue was purified by silica-gel column chromatography (hexane/ethyl acetate = 10/1→5/1) to obtain 11c (573 mg, 0.791 mmol, 22%) as a colorless oil.

^1^H-NMR (500 MHz, CDCl₃, rt): 1.27-1.37 (10H, m), 1.57-1.65 (4H, m), 2.03 (3H, s), 3.38-3.45 (1H, m), 3.56 (1H, d, J = 3.6, 9.6 Hz), 3.59-3.66 (3H, m), 3.73 (1H, d, J = 3.7, 10.6 Hz), 3.76-3.80 (1H, m), 3.99 (1H, t, J = 9.6 Hz), 4.04 (2H, t, J = 6.8 Hz), 4.46 (2H, d, J = 11.4 Hz), 4.61 (1H, d, J = 12.0 Hz), 4.65 (1H, d, J = 12.3 Hz), 4.76 (1H, d, J = 3.8 Hz), 4.77 (1H, d, J = 12.6 Hz), 4.82 (1H, d, J = 11.0 Hz), 4.83 (1H, d, J = 10.7 Hz), 4.99 (1H, d, J = 10.8 Hz), 7.12-7.15 (2H, m), 7.25-7.38 (18H, m) ppm; ^1^C-NMR (125 MHz, CDCl₃, rt): 20.93, 20.95, 25.82, 26.05, 28.50, 29.13, 29.26, 29.30, 29.33, 64.52, 68.10, 68.44, 70.00, 73.02, 73.37, 75.01, 75.59, 77.69, 80.02, 82.04, 96.80, 127.45, 127.57, 127.60, 127.71, 127.80, 127.83, 127.86, 127.92, 128.25, 128.28, 128.30, 137.88, 138.17, 138.28, 138.84, 171.11 ppm; HR ESI MS (positive): [M+Na]^+ found m/z 747.3845, C₄₅H₅₆O₈Na⁺ requires m/z 747.3873; IR (neat) v: 698, 737, 1072, 1242, 1363, 1455, 1737, 2856, 2927 cm⁻¹; [α]D²₅ +29.2° (c 1.00, CHCl₃)
2,3;4,6-di-O-isopropylidene-1-O-(9-(acetyloxy)nonyl)-α-D-glucopyranose (12c)

Compound 11c (573 mg, 0.791 mmol) was dissolved in methanol (10.0 mL) and THF (2.00 mL). The solution was adjusted to pH3 by addition of 1 M aq. hydrogen chloride. Palladium hydroxide on carbon (85.0 mg) was added to the solution and stirred for an hour under hydrogen atmosphere. The reaction mixture was passed through Celite® pad and evaporated. The residue was dissolved in 2,2-dimethoxypropane (10.0 mL), DMF (2.00 mL), and CSA (14.5 mg, 0.0625 mmol) was added and stirred for overnight at 70°C. After cooling to rt, TEA was added to the mixture and evaporated. The residue was purified by silica-gel column chromatography (hexane/ethyl acetate = 8/1→5/1) to obtain 12c (222 mg, 0.500 mmol, 63%) as a colorless oil.

1H-NMR (270 MHz, CDCl3, rt): 1.28-1.41 (10H, m), 1.45 (6H, s), 1.47 (3H, s), 1.55 (3H, s), 1.57-1.71 (4H, m), 2.04 (3H, s), 2.46-3.61 (3H, m), 3.66-3.77 (1H, m), 3.82-3.93 (3H, m), 4.05 (1H, t, J = 9.4 Hz), 4.05 (2H, t, J = 6.8 Hz), 5.12 (1H, d, J = 3.1 Hz) ppm; 13C-NMR (67.5 MHz, CDCl3, rt): 19.02, 20.84, 25.75, 25.86, 26.21, 26.70, 28.46, 28.93, 29.03, 29.12, 29.26, 29.30, 62.37, 64.54, 64.94, 68.64, 73.77, 74.00, 76.92, 97.86, 99.65, 111.35, 171.32 ppm; HR FD MS (positive): [M]+ found m/z 444.27274, C23H40O8 requires m/z 444.27232; IR (neat) ν: 840, 965, 1048, 1233, 1372, 1740, 2857, 2931, 2988 cm⁻¹; [α]D²³ + 55.7° (c 1.00, CHCl₃)

2,3;4,6-di-O-isopropylidene-1-O-(9-iodononyl)-α-D-glucopyranose (5c)

Compound 12c (131 mg, 0.295 mmol) was dissolved in methanol (2.00 mL) and THF (1.00 mL). Potassium carbonate (25.4 mg, 0.184 mmol) was added to the solution and stirred for 2.5 hrs at rt. The reaction mixture was diluted with water and extracted by ethyl acetate. The organic layer was washed with brine, dried over sodium sulfate and evaporated. The residue was dissolved in dichloromethane (3.0 mL) and triphenylphosphine (93.5 mg, 0.356 mmol), imidazole (32.4 mg, 0.476 mmol), NIS (99.0 mg, 0.440 mmol) was added. After stirring for overnight, the reaction mixture was diluted with chloroform, washed with sat. aq. sodium hydrogen carbonate and brine. The organic layer was dried over sodium sulfate and evaporated. The residue was purified by silica-gel column chromatography (hexane/ethyl acetate = 8/1→4/1) to obtain 5c (95.4 mg, 0.186 mmol, 63%) as a colorless oil.
$^1$H-NMR (270 MHz, CDCl$_3$, rt): 1.26-1.41 (10H, m), 1.45 (6H, s), 1.46 (3H, s), 1.55 (3H, s), 1.57-1.71 (2H, m), 1.76-1.88 (2H, m), 3.19 (2H, t, $J = 6.9$ Hz), 3.47-3.62 (3H, m), 3.63-3.95 (4H, m), 4.04 (1H, t, $J = 9.6$ Hz), 5.12 (1H, d, $J = 3.1$ Hz) ppm; $^{13}$C-NMR (67.5 MHz, CDCl$_3$, rt): 7.00, 19.00, 25.81, 26.20, 26.68, 28.28, 28.92, 29.07, 29.13, 29.26, 30.31, 33.38, 62.33, 64.90, 68.59, 73.73, 73.96, 76.88, 97.81, 99.61, 111.32 ppm; HR FD MS (positive): [M+H]$^+$ found m/z 513.17235, C$_{21}$H$_{38}$IO$_6$ requires m/z 513.17131; IR (neat) $\nu$: 840, 1049, 1076, 1116, 1203, 1372, 2855, 2929, 2988 cm$^{-1}$; $[\alpha]_D^{21} +49.1^\circ$ (c 1.00, CHCl$_3$)
Coupling of 4 and 5

Methyl

4-[4-(2,3;4,6-di-O-isopropylidene-α-D-glucopyranosyloxy)butyloxy]-2,3,2’,3’-tetra-O-(p-methoxybenzyl)-6-O-isopropylsilyl-α-acarviosin (13a)

Compound 5a (92.6 mg, 0.218 mmol) and 4 (62.6 mg, 0.0645 mmol) was dissolved in THF (0.50 mL), DMF (0.50 mL). The solution was cooled to 0°C, sodium hydride (30.5 mg, 1.27 mmol) was added and stirred for overnight under argon atmosphere at rt. The reaction mixture was diluted with water and extracted by ethyl acetate. The organic layer was washed with brine, dried over sodium sulfate and evaporated. The residue was purified by silica-gel column chromatography (hexane/ethyl acetate = 4/1→2/1) to obtain 13a (12.2 mg, 0.00948 mmol, 15%).

1H-NMR (500 MHz, CDCl3, rt): 1.01-1.13 (21H, m), 1.18 (3H, d, J = 6.0 Hz), 1.42-1.48 (9H, m), 1.54 (3H, s), 1.57-1.70 (4H, m), 2.41 (1H, t, J = 9.8 Hz), 3.35 (3H, s), 3.44-3.57 (7H, m), 3.60-3.66 (2H, m), 3.67-3.70 (1H, m), 3.75 (3H, s), 3.77 (3H, s), 3.789 (3H, s), 3.793 (3H, s), 3.81-3.90 (3H, m), 3.94 (1H, d, J = 6.5 Hz), 3.99 (1H, t, J = 4.5 Hz), 4.03 (1H, t, J = 9.4 Hz), 4.17 (1H, dd, J = 6.5, 9.5 Hz), 4.19 (1H, d, J = 13.8 Hz), 4.25 (1H, d, J = 13.8 Hz), 4.49 (1H, d, J = 3.6 Hz), 4.51 (1H, d, J = 11.7 Hz), 4.52 (1H, d, J = 11.7 Hz), 4.58 (1H, d, J = 10.9 Hz), 4.59-4.66 (3H, m), 4.79 (1H, d, J = 10.9 Hz), 4.93 (1H, d, J = 10.6 Hz), 5.10 (1H, d, J = 3.0 Hz), 5.92 (1H, d, J = 4.5 Hz), 6.76-6.86 (8H, m), 7.13-7.17 (4H, m), 7.21-7.23 (4H, m) ppm; 13C-NMR (125 MHz, CDCl3, rt): 11.86, 17.97, 18.78, 19.05, 26.24, 26.40, 26.73, 26.96, 28.94, 52.30, 54.93, 55.17, 55.17, 55.17, 55.17, 60.32, 62.36, 63.47, 64.99, 68.44, 68.52, 70.17, 72.26, 72.61, 73.77, 74.02, 74.02, 74.73, 76.92, 78.82, 79.97, 80.24, 81.05, 83.80, 97.87, 97.92, 99.67, 111.38, 113.70, 113.78, 113.79, 113.85, 123.11, 123.81, 128.81, 129.24, 129.60, 129.80, 130.46, 130.81, 131.34, 131.40, 140.37, 159.00, 159.23, 159.23, 159.23 ppm; HR FD MS (positive): [M+H]+ found m/z 1286.70214, C71H104NO18Si+ requires m/z 1286.70226; IR (neat) ν: 821, 1070, 1247, 1514, 2886, 2935 cm⁻¹; [α]D²¹ +30.8° (c 0.813, CHCl₃)
Methyl 4-[4-(α-D-glucopyranosyloxy)butyloxy]-α-acarviosin (6)

Compound 13a (12.2 mg, 9.48 µmol) was dissolved in methanol (0.200 mL). The solution was cooled to 0°C and trifluoroacetic acid (TFA, 0.50 mL) was added. The reaction mixture was stirred for 9 hrs at rt. After concentration of the mixture, the solution was charged on BondElut C18 column (Varian, Inc.) and eluted by 30% aq. methanol to obtain 6 (4.70 mg, 8.26 µmol, 87%) as a colorless oil.

\[ \text{1H-NMR (500 MHz, CD3OD, rt): } 1.42 (3H, d, J = 6.2 Hz), 1.69-1.79 (4H, m), 3.05 (1H, t, J = 10.2 Hz), 3.27-3.32 (1H, m), 3.42 (1H, dd, J = 3.8, 9.6 Hz), 3.44 (3H, s), 3.47-3.50 (1H, m), 3.52 (1H, dd, J = 3.6, 9.1 Hz), 3.57-3.72 (4H, m), 3.77-3.85 (3H, m), 3.91-4.11 (5H, m), 4.17 (1H, br s), 4.21 (1H, d, J = 15.0 Hz), 4.26 (1H, d, J = 15.0 Hz), 4.70 (1H, d, J = 3.6 Hz), 4.80 (1H, d, J = 3.8 Hz), 5.90 (1H, d, J = 2.7 Hz) ppm; \]

\[ \text{13C-NMR (67.5 MHz, CD3OD, rt): 18.60, 27.23, 28.00, 56.03, 57.26, 62.88, 63.00, 64.37, 64.99, 68.36, 68.91, 69.69, 71.30, 72.01, 73.19, 73.61, 73.79, 74.11, 75.16, 79.43, 100.22, 101.22, 116.21, 147.49 ppm; HR ESI MS (positive): [M+H]^+ \text{ found } m/z 570.2787, \text{ C}_{24}\text{H}_{44}\text{NO}_{14}^+ \text{ requires } m/z 570.2762; \text{ IR (neat) } \nu: 1049, 1138, 1202, 1672, 2937, 3359 \text{ cm}^{-1}; \alpha_D^{21} +79.7° \text{ (c 0.310, CH3OH) } \]

Methyl

4-[4-(2,3;4,6-di-O-isopropylidene-α-D-glucopyranosyloxy)hexyloxy]-2,3,2’,3’-tetra-O-(p-methoxybenzyl)-6-O-isopropylsilyl-α-acarviosin (13b)

Compound 5b (134 mg, 0.284 mmol) and 4 (71.5 mg, 0.0736 mmol) was dissolved in THF (0.50 mL), DMF (0.50 mL). The solution was cooled to 0°C, sodium hydride (34.7 mg, 1.45 mmol) was added and stirred for overnight under argon atmosphere at rt. The reaction mixture was diluted with water and extracted by ethyl acetate. The organic layer was washed with brine, dried over sodium sulfate and evaporated. The residue was purified by silica-gel column chromatography (hexane/ethyl acetate = 4/1→2/1) to obtain 13b (23.8 mg, 0.0181mmol, 25%).

\[ \text{1H-NMR (500 MHz, CDCl}_3, \text{ rt): 1.02-1.13 (21H, m), 1.19 (3H, d, J = 5.9 Hz), 1.28-1.39 } \]
(4H, m), 1.42-1.49 (12H, m), 1.55 (3H, s), 1.57-1.67 (4H, m), 2.42 (1H, t, \( J = 9.5 \) Hz), 3.35 (3H, s), 3.40-3.72 (10H, m), 3.75 (3H, s), 3.76 (3H, s), 3.78 (3H, s), 3.81-3.91 (3H, m), 3.94 (1H, d, \( J = 6.3 \) Hz), 3.99 (1H, brs), 4.04 (1H, t, \( J = 9.3 \) Hz), 4.15-4.20 (1H, m), 4.21 (1H, d, \( J = 14.5 \) Hz), 4.25 (1H, d, \( J = 14.5 \) Hz), 4.48-4.65 (7H, m), 4.78 (1H, d, \( J = 10.8 \) Hz), 4.93 (1H, d, \( J = 10.5 \) Hz), 5.11 (1H, d, \( J = 3.0 \) Hz), 5.93 (1H, brs), 6.75-6.86 (8H, m), 7.12-7.18 (4H, m), 7.21-7.27 (4H, m) ppm; 13C-NMR (125 MHz, CDCl3, rt): 11.90, 18.02, 18.83, 19.07, 25.97, 26.11, 26.26, 26.75, 28.97, 29.19, 29.41, 52.29, 53.72, 54.90, 55.09, 55.11, 55.12, 55.15, 60.28, 62.32, 63.36, 64.91, 68.36, 68.53, 70.07, 72.21, 72.53, 73.71, 73.94, 74.65, 76.85, 78.44, 79.85, 80.10, 80.93, 83.68, 97.73, 97.80, 99.55, 111.23, 113.54, 113.60, 113.68, 122.84, 128.64, 129.07, 129.43, 129.62, 130.26, 130.64, 131.17, 131.20, 140.13, 158.74, 158.97, 158.98, 159.24 ppm; HR FD MS (positive): [M]+ found m/z 1313.72327, C73H107NO18Si+ requires m/z 1313.72574; IR (neat) \( \nu \): 1075, 1247, 1457, 1514, 2865, 2935 cm\(^{-1}\); \([\alpha]\)_D\(^{22}\) +36.0° (c 0.992, CHCl\(_3\))

Methyl 4-[(6-\(\alpha\)-D-glucopyranosyloxy)hexyloxy]-\(\alpha\)-acarviosin (7)

Compound 13b (23.8 mg, 0.0181 mmol) was dissolved in methanol (0.66 mL). The solution was cooled to 0°C and trifluoroacetic acid (TFA, 1.70 mL) was added. The reaction mixture was stirred for 23 hrs at rt. After concentration of the mixture, the solution was charged on to BondElut C18 column (Varian, Inc.) and eluted by 30% aq. methanol to obtain 7 (10.9 mg, 0.0181 mmol, quant.) as a colorless oil.

1H-NMR (500 MHz, CD\(_3\)OD, rt): 1.39 (3H, d, \( J = 6.3 \) Hz), 1.41-1.47 (4H, m), 1.58-1.68 (4H, m), 3.03 (1H, t, \( J = 10.3 \) Hz), 3.25-3.31 (1H, m), 3.38 (1H, dd, \( J = 3.8, 9.8 \) Hz), 3.42 (3H, s), 3.42-3.47 (1H, m), 3.49 (1H, dd, \( J = 3.7, 9.1 \) Hz), 3.53-3.58 (1H, m), 3.60-3.68 (3H, m), 3.71-3.76 (1H, m), 3.77-3.81 (2H, m), 3.84-3.90 (1H, m), 3.92-3.99 (3H, m), 4.01-4.08 (1H, m), 4.13-4.25 (3H, m), 4.68 (1H, d, \( J = 3.7 \) Hz), 4.76 (1H, d, \( J = 3.8 \) Hz), 5.86 (1H, d, \( J = 2.5 \) Hz) ppm; 13C-NMR (67.5 MHz, CD\(_3\)OD, rt): 18.60, 27.01, 27.08, 30.45, 31.01, 56.03, 57.28, 62.80, 62.98, 64.36, 65.00, 68.36, 69.03, 69.68, 71.27, 71.96, 73.41, 73.63, 73.73, 74.10, 75.17, 79.46, 100.16, 101.22, 116.08, 147.49 ppm; HR ESI MS (positive): [M+H]\(^+\) found m/z 598.3065, C\(_{26}\)H\(_{48}\)NO\(_{14}\) requires m/z 598.3075; IR (neat) \( \nu \): 1049, 1144, 1202, 1671, 2671, 2937, 3365 cm\(^{-1}\); \([\alpha]\)_D\(^{24}\) +95.4° (c 0.500, CH\(_3\)OH)
Methyl 4-[4-(2,3;4,6-di-O-isopropylidene-α-D-glucopyranosyloxy)nonyloxy]-2,3,2',3'-tetra-O-(p-methoxybenzyl)-6-O-isopropylsilyl-α-acarviosin (13c)

Compound 5c (117 mg, 0.228 mmol) and 4 (72.8 mg, 0.0750 mmol) was dissolved in THF (0.50 mL), DMF (0.50 mL). The solution was cooled to 0°C, sodium hydride (35.9 mg, 1.50 mmol) was added and stirred for overnight under argon atmosphere at rt. The reaction mixture was diluted with water and extracted by ethyl acetate. The organic layer was washed with brine, dried over sodium sulfate and evaporated. The residue was purified by silica-gel column chromatography (hexane/ethyl acetate = 4/1→2/1) to obtain product containing 4. This was dissolved in dichloromethane (1.0 mL) and acetic anhydride (0.01 mL), TEA(0.02 mL) was added and stirred for 3.5 hrs at rt. The reaction mixture was diluted with chloroform, washed with sat. aq. sodium hydrogen carbonate and brine, and dried over sodium sulfate. After evaporation, the residue was purified by silica-gel column chromatography (hexane/ethyl acetate = 4/1→5/2) to obtain 13c (25.6 mg, 0.0199 mmol, 25%).

$^1$H-NMR (500 MHz, CDCl$_3$, rt): 1.02-1.13 (21H, m), 1.19 (3H, d, $J= 6.3$ Hz), 1.23-1.37 (10H, m), 1.44 (3H, s), 1.45 (3H, s), 1.46 (3H, s), 1.46-1.54 (2H, m), 1.55 (3H, s), 1.58-1.68 (2H, m), 2.42 (1H, t, $J= 9.6$ Hz), 3.35 (3H, s), 3.40-3.59 (8H, m), 3.64 (1H, t, $J= 9.6$ Hz), 3.68-3.73 (1H, m), 3.74 (3H, s), 3.76 (3H, s), 3.78 (3H, s), 3.79 (3H, s), 3.80-3.91 (3H, m), 3.94 (1H, d, $J= 6.7$ Hz), 3.99 (1H, t, $J= 4.7$ Hz), 4.05 (1H, t, $J= 9.3$ Hz), 4.19 (1H, dd, $J= 6.7$, 9.3 Hz), 4.24 (2H, br s), 4.49 -4.66 (7H, m), 4.77 (1H, d, $J= 10.7$ Hz), 4.93 (1H, d, $J= 10.8$ Hz), 5.12 (1H, d, $J= 3.1$ Hz), 5.94 (1H, d, $J= 4.7$ Hz), 6.75-6.86 (8H, m), 7.14-7.18 (4H, m), 7.21-7.28 (4H, m) ppm; $^{13}$C-NMR (125 MHz, CDCl$_3$, rt): 11.87, 17.98, 18.80, 19.04, 25.94, 26.22, 26.24, 26.72, 28.93, 29.27, 29.35, 29.49, 30.31, 52.26, 54.85, 55.04, 55.06, 55.07, 55.09, 60.22, 62.30, 63.31, 64.86, 68.32, 68.60, 70.00, 72.19, 72.49, 73.68, 73.88, 73.92, 74.60, 76.83, 78.30, 79.81, 80.06, 80.90, 83.64, 97.71, 97.76, 99.50, 111.18, 113.50, 113.56, 113.64, 122.76, 128.60, 129.04, 129.41, 129.58, 130.22, 130.62, 131.15, 131.17, 140.11, 158.71, 158.93, 158.95, 159.21 ppm; HR FD MS (positive): [M]$^+$ found m/z 1355.77361, C$_{76}$H$_{113}$NO$_{18}$Si$^+$ requires m/z 1355.77269; IR (neat) ν: 1075, 1247, 1514, 2864, 2932 cm$^{-1}$; [α]$_D$$^{21}$ +39.7° (c 0.985, CHCl$_3$)
Methyl 4-[9-(α-D-glucopyranosyloxy)nonyloxy]-α-acarviosin (8)

Compound 13c (25.4 mg, 0.0187 mmol) was dissolved in methanol (0.66 mL). The solution was cooled to 0°C and trifluoroacetic acid (TFA, 1.70 mL) was added. The reaction mixture was stirred for 23 hrs at rt. After concentration of the mixture, the solution was charged on BondElut C18 column (Varian, Inc.) and eluted by 30% aq. methanol to obtain 8 (12.0 mg, 0.0188 mmol, quant.) as a colorless oil.

1H-NMR (500 MHz, CD3OD, rt): 1.30-1.41 (13H, m), 1.55-1.67 (4H, m), 3.03 (1H, t, J = 10.3 Hz), 3.25-3.30 (1H, m), 3.38 (1H, dd, J = 3.7, 9.8 Hz), 3.41 (3H, s), 3.42-3.46 (1H, m), 3.49 (1H, dd, J = 3.7, 9.2 Hz), 3.54-3.58 (1H, m), 3.69-3.75 (4H, m), 3.76-3.81 (2H, m), 3.92-4.00 (3H, m), 4.02-4.08 (1H, m), 4.13-4.25 (3H, m), 4.68 (1H, d, J = 3.7 Hz), 4.76 (1H, d, J = 3.7 Hz), 5.87 (1H, J = 2.5 Hz) ppm; 13C-NMR (125 MHz, CD3OD, rt): 18.66, 27.17, 27.25, 30.42, 30.44, 30.58, 31.16, 55.99, 57.29, 62.68, 62.93, 64.33, 64.93, 68.25, 69.10, 69.65, 71.15, 71.82, 73.41, 73.56, 73.61, 74.01, 75.13, 79.34, 100.06, 101.07, 115.94, 147.20 ppm; HR ESI MS (positive): [M+H]⁺ found m/z 640.3530, C29H54NO14⁺ requires m/z 640.3544; IR (neat) ν: 1040, 1140, 1200, 1671, 2857, 2930, 3364 cm⁻¹; [α]D²¹ +80.2° (c 0.500, CH₃OH)

Methyl 2,3,2′,3′-tetra-O-p-methoxybenzyl-4-O-methyl-6-O-triisopropylsilyl-α-acarviosin (14)

Compound 4 (62.8 mg, 0.0647 mmol) was dissolved in THF (0.50 mL), DMF (0.50 mL). The solution was cooled to 0°C, sodium hydride (34.7 mg, 1.45 mmol), iodomethane (0.05 mL) was added and stirred for four hrs under argon atmosphere at rt. The reaction mixture was diluted with water and extracted by ethyl acetate. The organic layer was washed with brine, dried over sodium sulfate and evaporated. The residue was purified by silica-gel column chromatography (hexane/ethyl acetate = 2/1) to obtain 14 (48.1 mg, 0.0488 mmol, 75%) as a colorless oil.

1H-NMR (500 MHz, CDC13, rt): 1.02-1.15 (21H, m), 1.17 (3H, d, J = 6.1 Hz), 2.46 (1H, t, J = 9.5 Hz), 3.35 (3H, s), 3.40 (3H, s), 3.44-3.50 (3H, m), 3.63 (1H, t, J = 9.3 Hz), 3.75 (3H, s), 3.77 (3H, s), 3.787 (3H, s), 3.79 (3H, s), 3.91 (1H, d, J = 6.9 Hz), 4.01 (1H, t, J = 4.7 Hz), 4.15-4.21 (2H, m), 4.27 (1H, d, J = 13.6 Hz), 4.49 (1H, d, J = 3.3 Hz), 4.50-4.67 (6H, m), 4.79 (1H, d, J = 10.6 Hz), 4.94 (1H,
d, J = 10.7 Hz), 5.93 (1H, d, J = 5.4 Hz), 6.77-6.87 (8H, m), 7.13-7.28 (8H, m) ppm; $^{13}$C-NMR (67.5 MHz, CDCl$_3$, rt): 11.81, 17.93, 18.75, 52.25, 54.89, 55.10, 55.11, 55.13, 55.15, 55.16, 57.76, 60.12, 63.38, 68.37, 72.32, 72.56, 74.03, 74.69, 78.29, 79.86, 81.01, 81.50, 83.87, 97.87, 113.68, 113.73, 113.75, 113.81, 123.57, 128.78, 129.23, 129.64, 129.76, 130.40, 130.76, 131.30, 131.33, 140.23, 158.97, 159.21, 159.46 ppm; HR ESI MS (positive): [M+H]$^+$ found m/z 986.5445, C$_{56}$H$_{80}$NO$_{12}$Si$^+$ requires m/z 986.5450; IR (neat) $\nu$: 821, 1078, 1173, 1249, 1302, 1458, 1514, 1614, 2865, 2938 cm$^{-1}$; $[\alpha]_{D}^{23}$ +37.9° (c 0.500, CHCl$_3$)

Methyl 4- O-methyl $\alpha$-acarviosin (9) の合成

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\text{Compound 14 (40.6 mg, 0.0412 mmol) was dissolved in methanol (0.67 mL). The solution was cooled to 0°C and TFA (1.70 mL) was added. The reaction mixture was stirred for 38 hrs at rt. After evaporation the residue was purified by silica-gel column chromatography (ethyl acetate/methanol = 16/1) to obtain 9 (13.2 mg, 0.0378 mmol, 92%) as a colorless oil. $^1$H-NMR (500 MHz, CD$_3$OD, rt): 1.29 (3H, d, J = 6.2 Hz), 2.34 (1H, t, J = 9.7 Hz), 3.36 (3H, s), 3.40 (1H, dd, J = 3.7, 9.1 Hz), 3.46-3.58 (7H, m), 3.72 (1H, d, J = 6.7 Hz), 3.91 (1H, dd, J = 6.7, 9.1 Hz), 4.03 (1H, d, J = 13.6 Hz), 4.13 (1H, d, J = 13.6 Hz), 4.59 (1H, d, J = 3.7 Hz), 5.87 (1H, d, J = 4.7 Hz) ppm; $^{13}$C-NMR (125 MHz, CD$_3$OD, rt): 18.67, 55.50, 57.69, 58.98, 63.15, 67.31, 69.53, 72.55, 72.93, 74.63, 74.91, 82.30, 101.20, 124.85, 140.93 ppm; HR ESI MS (positive): [M+H]$^+$ found m/z 350.1818, C$_{15}$H$_{28}$NO$_8$ requires m/z 350.1815; IR (neat) $\nu$: 1055, 1137, 1204, 1673, 2930, 3336 cm$^{-1}$; $[\alpha]_{D}^{23}$ +129.3° (c 0.500, CH$_3$OH) }

Reference

Supplementary material 2

Synthesis and study of the pancreatic α-amylase inhibitory activity of methyl acarviosin and its derivatives

Eisuke Kato,* Fumiaki Chikahisa, and Jun Kawabata

Laboratory of Food Biochemistry, Division of Applied Bioscience, Graduate School of Agriculture, Hokkaido University, Kita-ku, Sapporo, Hokkaido 060-8589, Japan

*Corresponding author:

e-mail: eikato@chem.agr.hokudai.ac.jp, Tel/Fax: +81-11-706-2496.
NMR spectrum of the synthetic compounds

Isopropylidene protected methyl α-acarviosin
Isopropylidene protected methyl β-acarviosin

This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmproc/

Acquisition Time (sec): 11,790
File Name: S2-3

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Isopropylidene beta_Moxy VerticalScaleFactor = 1

This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmproc/

Acquisition Time (sec): 11,790
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Sweep Time (sec) 0.5
Temperature (deg. C) 293.15
Sweep Width (Hz) 1.118e+03
Spectrum Offset (Hz) 182023

Vertical Scale Factor = 1

1H spectrum

This report was created by ACD/NMR Processor Academic Edition. For more information go to www.acdlabs.com/nmrmrc

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13C spectrum

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