

Potent chemical chaperone compounds for G_{M1}-gangliosidosis: N-substituted (+)-conduramine F-4 derivatives

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Electronic Supplementary Information

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1. General Methods.

The optical rotations were measured with a HORIBA SEPA-200 high sensitivity polarimeter (Horiba, Kyoto, Japan). The ^1H NMR spectra were recorded in solutions of CDCl_3 and CD_3OD with a JEOL JNM-ECS 400 (400 MHz) instrument; the $\delta(\text{H})$ values are reported in parts per million relative to the residual solvent ^1H signal (CDCl_3 , $\delta(\text{H})$ 7.24; CD_3OD , $\delta(\text{H})$ 3.30) unless otherwise noted. The ^{13}C NMR spectra (100 MHz) were recorded on the same instrument as the ^1H spectra; $\delta(\text{C})$ is reported in ppm relative to the C signal of the solvent (CDCl_3 , $\delta(\text{C})$ 77.0; CD_3OD , $\delta(\text{C})$ 49.0) unless otherwise noted. High-resolution electron spray ionization mass spectra (HR-ESI-MS) were obtained on an LTQ Orbitrap mass spectrometer (Thermo Fisher Scientific, Bremen, Germany). The reagents and solvents were purchased from commercial suppliers. TLC was performed using silica gel 60 F-254 (E. Merck, Darmstadt, Germany). The silica gel used for chromatography was Wakogel C-300 (Wako Junyaku Kogyo Co., Osaka, Japan, 200-300 mesh). The organic solutions were dried over anhydrous Na_2SO_4 and concentrated with a rotary evaporator under reduced pressure.

2. General Procedures for Biological Assays.

2.1. Cell culture and chaperone test

Normal human skin fibroblasts and mouse β -galactosidase-gene knockout fibroblast cell lines stably expressing human normal or mutant R201C β -galactosidase enzymes were cultured in Dulbecco's modified Eagle's medium (Wako, Tokyo, Japan) with 10% fetal bovine serum (Biowest, Nuaille, France). For the chaperone test, the mouse fibroblasts were cultured in the medium with or without chaperone compounds for 48 h, as previously described ^{1, 2}. The lysates were assessed for β -galactosidase activity as

described below.

2.2. Lysosomal β -galactosidase assay

The lysosomal β -galactosidase enzyme activities in the cell lysates were measured using 4-methylumbelliferone (4-MU)-conjugated β -D-galactoside (Sigma, St. Louise, MO), as previously described^{1, 2}. Briefly, the cell lysates in a Triton X-100 solution (0.1% in distilled water) were mixed with the 4-MU substrate in 0.1 mol/l citrate buffer (pH 4.5); this mixture was incubated at 37°C for 30 min. The reaction was terminated by adding 0.2 mol/l glycine-NaOH buffer (pH 10.7), and the liberated 4-MU was measured with a fluorescence plate reader (ex. 340 nm, em. 460 nm; Infinite F-500, Tecan, Kawasaki, Japan). The activities were normalized using the protein concentrations in lysates.

2.3. Inhibition of normal human β -galactosidase, β -glucosidase, and α -galactosidase *in vitro*

The inhibition activities of compounds against normal human β -galactosidase, β -glucosidase, and α -galactosidase were evaluated using cell lysates from normal human fibroblasts². Lysates from human normal fibroblasts in Triton X-100 were mixed with the corresponding 4-MU substrate and each indicated concentration of the compounds. The mixture was incubated at 37°C for 30 min, and the liberated 4-MU was measured as described above.

2.4. Inhibitory activity toward commercially available glycosidases.

The compounds were assayed for enzyme inhibitory activity (IC_{50}) against commercially available bovine liver β -galactosidase (Sigma-Aldrich) or almond β -

glucosidase (Sigma-Aldrich). Various concentrations (final concentrations: 0, 0.01, 0.1, 1, 10, 100, 1000 μM) of the compounds in water (20 μL) were added to the assay mixture with an aqueous 10 mM *p*-nitrophenyl- β -D-galactopyranoside (Sigma-Aldrich) solution (5 μL) and a 200 mM sodium phosphate buffer (5 μL , pH 7.0) for the β -galactosidase assay or an aqueous 10 mM *p*-nitrophenyl- β -D-glucopyranoside (Sigma-Aldrich) solution (5 μL) and a 300 mM sodium acetate buffer (5 μL , pH 5.0) for the β -glucosidase assay, respectively. Afterwards, 20 μL of bovine liver β -galactosidase in sodium phosphate buffer (0.1 U/mL) or 20 μL of almond β -glucosidase in sodium acetate buffer (0.5 U/mL), respectively, was added to the mixture, which was incubated at 37°C. After 30 min, the reaction was quenched by adding a 200 mM aqueous sodium carbonate solution (150 μL), and the absorbance of the liberated *p*-nitrophenol was measured at 410 nm with a Sunrise-Basic Tecan microplate reader (Tecan, Salzburg, Austria). The percentage of inhibition was calculated.

2.5. LDH assay.

Cytotoxic effect of conduramine F-4 derivatives on cultured normal human fibroblasts was assessed by lactate dehydrogenase (LDH) assay. Briefly, normal human fibroblasts were cultured with or without treatment of chaperone compounds for 24h, and the cultured medium was collected. LDH activity in the medium was measured using LDH Cytotoxic Test Wako (WAKO, Tokyo, Japan).

References:

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3. Experimental Synthetic Procedures.

3.1. 1,2:3,4-di-*O*-Isopropylidene derivative of (1*R*,2*S*,3*S*,4*S*,5*R*)-1,2,3,4,5-pentahydroxycyclohexane (4): To a mixture of (+)-*proto*-quercitol (**2**) (1.50 g, 9.15 mmol) and 2,2-dimethoxypropane (11 mL, 91 mmol) in DMF (45 mL) was added *p*-TsOH·H₂O (435 mg, 2.29 mmol) portion-wise, and the mixture was stirred for 20 h at rt. The reaction solution was neutralized with NaHCO₃, filtered and concentrated. To the residue was added water, which was extracted three times with EtOAc. The combined organic layers were concentrated, and the residue was chromatographed on a silica gel column (3/1 hexane/EtOAc) to give **3** (2.00 g, 90%) as a white amorphous solid: $[\alpha]_{\text{D}}^{23} +27$ (*c* 1.0, MeOH); $R_{\text{F}} = 0.43$ (1:1 hexane/EtOAc); ¹H-NMR (400 MHz, CD₃OD): δ 1.34 (s, 3H, CMe₂), 1.38 (s, 6H, CMe₂), 1.46 (s, 3H, CMe₂), 1.86 (ddd, 1H, $J_{5,6\text{ax}} = 4.8$, $J_{1,6\text{ax}} = 11.7$, $J_{6\text{ax},6\text{eq}} = 12.8$ Hz, H-6ax), 2.07 (dddd, 1H, $J_{4,6\text{eq}} = 1.0$, $J_{5,6\text{eq}} = 3.6$, $J_{1,6\text{eq}} = 4.8$, $J_{6\text{ax},6\text{eq}} = 12.8$ Hz, H-6eq), 3.50 (dd, 1H, $J_{2,3} = 8.4$, $J_{1,2} = 10.0$, H-2), 3.74 (ddd, 1H, $J_{1,6\text{eq}} = 4.8$, $J_{1,2} = 10.0$, $J_{1,6\text{ax}} = 11.7$ Hz, H-1), 4.19-4.27 (m, 3H, H-3, H-4, H-5), assigned by H-H COSY; ¹³C-NMR (100 MHz, CD₃OD): δ 26.05, 27.19, 27.29, 28.41,

33.75, 69.01, 73.56, 77.85, 81.20, 83.46, 110.393, 111.70; HR-ESI-MS: 267.1202 ($C_{12}H_{20}O_5Na^+$, $[M+Na]^+$; calcd 267.1203).

3.2. 2,3:4,5-di-*O*-Isopropylidene derivative of (1*R*,2*R*,3*R*,4*S*,5*R*)-2,3,4,5-tetrahydrocyclohexyl methanesulfonate (5): Compound **4** (597 mg, 2.44 mmol) and triethylamine (1.35 mL, 9.76 mmol) were dissolved in CH_2Cl_2 (6 ml). After the solution was cooled in an ice bath, methanesulfonyl chloride (0.233 mL, 3.06 mmol) in CH_2Cl_2 (6 mL) was added dropwise. The mixture was stirred for 2 h at rt. While cooling in an ice bath, the reaction was quenched with MeOH and the solution was concentrated. To the residue was added water, which was extracted three times with EtOAc. The combined organic layer was dried and concentrated, and the residue was purified via silica gel column chromatography (9/1→4/1 hexane/EtOAc) to give compound **5** (762 mg, 97%) as a white amorphous powder: $[\alpha]_D^{23}$ -31 (*c* 1.0, MeOH); R_F = 0.75 (1:1 hexane/EtOAc); 1H -NMR (400 MHz, CD_3OD): δ 1.36 (s, 3H, CMe_2), 1.40 (s, 6H, CMe_2), 1.50 (s, 3H, CMe_2), 2.13-2.22 (m, 1H, H-6eq), 2.25-2.32 (m, 1H, H-6ax), 3.65 (ddd, 1H, $J_{2,4} = 2.4$, $J_{2,3} = 8.0$, $J_{1,2} = 10.1$, H-2), 3.77 (dddd, 1H, $J_{1,3} = 2.4$, $J_{1,6eq} = 6.2$, $J_{1,2} = 10.4$, $J_{1,6ax} = 10.4$ Hz, H-1), 4.34-4.38 (m, 1H, H-3), 4.43 (ddd, 1H, $J_{2,4} = 2.4$, $J_{3,4} = 6.2$, $J_{4,5} = 6.2$, H-4), 5.04-5.09 (m, 1H, H-5), assigned by H-H COSY; ^{13}C -NMR (100 MHz, CD_3OD): δ 25.61, 27.19, 27.27, 27.90, 32.42, 38.36, 72.72, 77.22, 78.54, 79.53, 81.91, 111.63, 112.98; HR-ESI-MS: 345.0971 ($C_{13}H_{22}O_7NaS^+$, $[M+Na]^+$; calcd 345.0978).

3.3. 1,2:3,4-di-*O*-Isopropylidene derivative of (1*R*,2*S*,3*S*,4*S*)-1,2,3,4-tetrahydrocyclohex-5-ene (6): DBU (1.18 mL, 7.75 mmol) was added to a solution

of **5** (500 mg, 1.55 mmol) in toluene (7 mL). The mixture was refluxed for 8 h, cooled to rt, and evaporated. The residue was purified on a silica gel column (95/5 hexane/EtOAc) to give **6** (267 mg, 76%) as a white powder: $[\alpha]_{\text{D}}^{23} +15$ (*c* 1.0, CHCl₃); $R_{\text{F}} = 0.45$ (4:1 hexane/EtOAc); ¹H-NMR (400 MHz, CDCl₃): δ 1.35, 1.42, 1.43, 1.49 (4s, each 3H, CMe₂), 3.51 (dd, 1H, $J_{1,2} = 9.0$, $J_{2,3} = 9.0$ Hz, H-2), 4.03 (br d, 1H, $J_{1,2} = 8.9$ Hz, H-1), 4.36 (dd, 1H, $J_{3,4} = 7.7$, $J_{2,3} = 9.2$ Hz, H-3), 4.79 (br d, 1H, $J_{3,4} = 7.6$ Hz, H-4), 5.78 (ddd, 1H, $J_{1,5} = 2.6$, $J_{4,5} = 2.6$, $J_{5,6} = 9.9$ Hz, H-5), 6.16 (ddd, 1H, $J_{1,6} = 1.5$, $J_{4,6} = 1.5$, $J_{5,6} = 9.9$ Hz, H-6), assigned by H-H COSY; ¹³C-NMR (100 MHz, CDCl₃): δ 24.59, 26.74, 26.79, 27.25, 74.58, 74.66, 74.84, 81.42, 110.50, 112.69, 125.59, 128.48; HR-ESI-MS: 249.1095 (C₁₂H₁₈O₄Na⁺, [M+Na]⁺; calcd 249.1097).

3.4. 3,4-*O*-Isopropylidene derivative of (1*R*,2*S*,3*S*,4*S*)-1,2,3,4-tetrahydrocyclohex-5-ene (7): Pyridinium-*p*-toluenesulfonate (62 mg, 0.25 mmol) was slowly added portionwise to an ice-cooled solution of compound **6** (561 mg, 2.48 mmol) in methanol (25 mL). The mixture was kept for 15 h at 4°C and then neutralized with Et₃N. The solution was concentrated and the residue was purified by silica gel column chromatography (1/2 hexane/EtOAc) to yield **7** (421 mg, 91%) as a white powder: $[\alpha]_{\text{D}}^{23} +66$ (*c* 1.0, MeOH); $R_{\text{F}} = 0.11$ (1:1 hexane/EtOAc); ¹H-NMR (400 MHz, CD₃OD): δ 1.36, 1.46 (2s, each 3H, CMe₂), 3.39 (dd, 1H, $J_{1,2} = 9.2$, $J_{2,3} = 9.2$ Hz, H-2), 3.92-3.95 (m, 1H, H-1), 4.02 (dd, 1H, $J_{3,4} = 6.4$, $J_{2,3} = 9.2$ Hz, H-3), 4.63-4.66 (m, 1H, H-4), 5.79-5.80 (m, 2H, H-5, H-6); ¹³C-NMR (100 MHz, CD₃OD): δ 26.06, 28.52, 71.69, 74.05, 75.84, 79.39, 111.22, 124.17, 135.96; HR-ESI-MS: 209.0786 (C₉H₁₄O₄Na⁺, [M+Na]⁺; calcd 209.0784).

3.5. 1,2-Epoxy-3,4-*O*-isopropylidene derivative of (1*S*,2*S*,3*S*,4*S*)-3,4-dihydroxycyclohex-5-ene (8): To a stirred solution of **7** (669 mg, 3.59 mmol) in CH₂Cl₂ (36 mL) was slowly added a solution of Martin's sulfurane (2.90 g, 4.31 mmol) in CH₂Cl₂ (18 mL) at rt. The mixture was stirred for 0.5 h at rt before it was diluted with CHCl₃ and washed with a 20% KOH aqueous solution. The organic layer was separated, and the aqueous layer was re-extracted with CHCl₃. The combined organic layer was dried and evaporated; the residue was chromatographed on a silica gel column (9/1 hexane/EtOAc) to give **8** (418 mg, 69%) as a colorless oil: $[\alpha]_{\text{D}}^{23} +58$ (*c* 1.0, MeOH); *R*_F = 0.41 (4:1 hexane/EtOAc); ¹H-NMR (400 MHz, CD₃OD): δ 1.34, 1.36 (2s, each 3H, CMe₂), 3.31-3.33 (1H, H-1), 3.49 (dd, 1H, *J*_{2,3} = 1.8, *J*_{1,2} = 3.8 Hz, H-2), 4.39 (ddd, 1H, *J*_{2,4} = 2.0, *J*_{4,5} = 2.0 *J*_{3,4} = 6.8 Hz, H-4), 4.76 (ddd, 1H, *J*_{1,3} = 1.6, *J*_{2,3} = 1.6, *J*_{3,4} = 6.8 Hz, H-3), 5.74 (br d, 1H, *J*_{5,6} = 10.4 Hz, H-5). 6.05 (ddd, 1H, *J*_{2,6} = 1.6, *J*_{1,6} = 4.0, *J*_{5,6} = 10.4 Hz, H-6), assigned by H-H COSY; ¹³C-NMR (100 MHz, CD₃OD): δ 26.12, 28.13, 47.37, 50.14, 72.03, 72.26, 111.60, 124.76, 133.02; HR-ESI-MS: 191.0676 (C₉H₁₂O₃Na⁺, [M+Na]⁺; calcd 191.0679).

3.6. *N*-substituted conduramine F-4 derivatives (3a, 3c, 3d, 3g, 3h, 3i, 3m, and 3n, representative compounds with excellent chaperone activities): For **3a**: A solution of **8** (211 mg, 1.26 mmol) and *n*-octylamine (624 μ L, 3.77 mmol) in acetonitrile (12 mL) was added to a sealed tube, and the mixture was allowed to stand for 2 days at 60°C. The reaction solution was cooled to rt and evaporated. The residue was chromatographed on a silica gel column (99/1 \rightarrow 98/2 CHCl₃/MeOH). The purified product was dissolved in 20 mL of 1 M HCl/THF (1/1) and stirred for 2 h at rt. The reaction mixture was co-evaporated with EtOH to give **3a** [374 mg, quantitative

(101%)] as an amorphous white powder: $[\alpha]_{\text{D}}^{23} +4$ (*c* 1.0, MeOH); $R_{\text{F}} = 0.66$ (6:3:1 CHCl₃/MeOH/AcOH); ¹H-NMR (400 MHz, CD₃OD): δ 0.89 (t, 3H, $J_{7,8'} = 7.0$ Hz, H-8'), 1.29-1.41 (10H, H-3', 4', 5', 6', and 7'), 1.68-1.76 (m, 2H, H-2'), 3.06-3.14 (m, 2H, H-1'), 3.60 (dd, 1H, $J_{3,4} = 4.0$, $J_{2,3} = 9.2$, Hz, H-3), 3.69 (br d, 1H, $J_{1,2} = 7.6$ Hz, H-1), 3.96 (dd, 1H, $J_{1,2} = 7.6$, $J_{2,3} = 9.4$ Hz, H-2), 4.25 (dd, 1H, $J_{3,4} = 4.4$, $J_{4,5} = 4.4$ Hz, H-4), 5.83 (dd, 1H, $J_{1,6} = 2.2$, $J_{5,6} = 10.2$ Hz, H-6). 6.11 (ddd, 1H, $J_{1,5} = 2.2$, $J_{4,5} = 4.8$, $J_{5,6} = 10.2$ Hz, H-5); ¹³C-NMR (100 MHz, CD₃OD): δ 14.39, 23.65, 27.39, 27.62, 30.15, 32.86, 46.13, 61.14, 67.17, 68.12, 72.50, 123.44, 134.81; HR-ESI-MS: 258.2060 (C₁₄H₂₈O₃N⁺, [M+H]⁺; calcd 258.2064).

3c: Viscous syrup: Yield = 46 mg [quantitative (102%)]; $[\alpha]_{\text{D}}^{23} +10$ (*c* 1.0, MeOH); ¹H-NMR (400 MHz, CD₃OD): δ 0.89 (t, 3H, $J_{4',5'} = 7.0$ Hz, H-5'), 1.37-1.41 (4H, H-3' and 4'), 1.71-1.78 (m, 2H, H-2'), 3.05-3.17 (m, 2H, H-1'), 3.61 (dd, 1H, $J_{3,4} = 4.4$, $J_{2,3} = 9.0$ Hz, H-3), 3.71 (br d, 1H, $J_{1,2} = 8.0$ Hz, H-1), 3.98 (dd, 1H, $J_{1,2} = 7.6$, $J_{2,3} = 9.2$ Hz, H-2), 4.26 (dd, 1H, $J_{3,4} = 4.4$, $J_{4,5} = 4.4$ Hz, H-4), 5.85 (dd, 1H, $J_{1,6} = 2.4$, $J_{5,6} = 10.0$ Hz, H-6). 6.11 (ddd, 1H, $J_{1,5} = 2.0$, $J_{4,5} = 4.8$, $J_{5,6} = 10.1$ Hz, H-5); ¹³C-NMR (100 MHz, CD₃OD): δ 14.13, 23.18, 27.05, 29.71, 46.11, 61.14, 67.15, 68.09, 72.48, 123.50, 134.74; HR-ESI-MS: 216.1592 (C₁₁H₂₂O₃⁺, [M+H]⁺; calcd 216.1594).

3d: White powder: Yield = 27 mg (85%); $[\alpha]_{\text{D}}^{23} +8$ (*c* 1.0, MeOH); ¹H-NMR (400 MHz, CD₃OD): δ 0.93 (t, 3H, $J_{4',5'} = 7.2$ Hz, H-6'), 1.36-1.42 (6H, H-3', 4' and 5'), 1.66-1.74 (m, 2H, H-2'), 3.04-3.15 (m, 2H, H-1'), 3.58 (dd, 1H, $J_{3,4} = 4.4$, $J_{2,3} = 9.8$ Hz, H-3), 3.66 (ddd, 1H, $J_{1,5} = 2.2$, $J_{1,6} = 2.2$, $J_{1,2} = 7.6$ Hz, H-1), 3.93 (dd, 1H, $J_{1,2} = 7.6$, $J_{2,3} = 9.2$ Hz, H-2), 4.26 (dd, 1H, $J_{3,4} = 4.4$, $J_{4,5} = 4.4$ Hz, H-4), 5.79 (dd, 1H, $J_{1,6} = 2.2$, $J_{5,6} = 10.2$

Hz, H-6). 6.12 (ddd, 1H, $J_{1,5} = 2.2$, $J_{4,5} = 4.6$, $J_{5,6} = 10.1$ Hz, H-5); ^{13}C -NMR (100 MHz, CD_3OD): δ 14.27, 23.45, 27.31, 27.35, 32.37, 46.12, 61.14, 67.16, 68.11, 72.50, 123.44, 134.82; HR-ESI-MS: 230.1750 ($\text{C}_{12}\text{H}_{24}\text{O}_3\text{N}^+$, $[\text{M}+\text{H}]^+$; calcd 230.1751).

3g: Amorphous white powder: Yield = 27 mg (64%); $[\alpha]_{\text{D}}^{23} +13$ (c 1.0, MeOH); ^1H -NMR (400 MHz, CD_3OD): δ 1.05 (d, 6H, $J_{2',3'} = 6.4$ Hz, H-3'), 2.04 (dq, 2H, $J_{1',2'} = 6.6$, $J_{2',3'} = 6.6$ Hz, H-2'), 2.95 (d, 2H, $J_{1',2'} = 6.8$ Hz, H-1'), 3.61 (dd, 1H, $J_{3,4} = 4.4$, $J_{2,3} = 9.0$ Hz, H-3), 3.72 (br d, 1H, $J_{1,2} = 7.2$ Hz, H-1), 3.98 (dd, 1H, $J_{1,2} = 7.6$, $J_{2,3} = 9.2$ Hz, H-2), 4.26 (dd, 1H, $J_{3,4} = 4.4$, $J_{4,5} = 4.4$ Hz, H-4), 5.82 (dd, 1H, $J_{1,6} = 2.4$, $J_{5,6} = 10.0$ Hz, H-6). 6.13 (ddd, 1H, $J_{1,5} = 2.4$, $J_{4,5} = 4.8$, $J_{5,6} = 10.2$ Hz, H-5); ^{13}C -NMR (100 MHz, CD_3OD): δ 20.23, 20.35, 27.38, 52.75, 61.52, 67.22, 67.71, 72.61, 123.35, 135.14; HR-ESI-MS: 202.1435 ($\text{C}_{10}\text{H}_{20}\text{O}_3\text{N}^+$, $[\text{M}+\text{H}]^+$; calcd 202.1438).

3h: Amorphous white powder: Yield = 26 mg (82%); $[\alpha]_{\text{D}}^{23} +7$ (c 1.0, MeOH); ^1H -NMR (400 MHz, CD_3OD): δ 0.94 (t, 6H, $J_{3',4'} = 7.2$ Hz, H-4'), 1.42-1.50 (m, 2H, H-3'), 1.67 (tt, 1H, $J_{2',3'} = 6.4$, $J_{1',2'} = 6.8$ Hz, H-2'), 3.02 (d, 2H, $J_{1',2'} = 7.2$ Hz, H-1'), 3.62 (dd, 1H, $J_{3,4} = 4.0$, $J_{2,3} = 9.2$ Hz, H-3), 3.74 (br d, 1H, $J_{1,2} = 7.6$ Hz, H-1), 4.00 (dd, 1H, $J_{1,2} = 7.6$, $J_{2,3} = 9.2$ Hz, H-2), 4.26 (dd, 1H, $J_{3,4} = 4.4$, $J_{4,5} = 4.4$ Hz, H-4), 5.82 (dd, 1H, $J_{1,6} = 2.4$, $J_{5,6} = 10.0$ Hz, H-6). 6.13 (ddd, 1H, $J_{1,5} = 2.4$, $J_{4,5} = 4.4$, $J_{5,6} = 10.1$ Hz, H-5); ^{13}C -NMR (100 MHz, CD_3OD): δ 10.55, 10.59, 24.06, 24.11, 39.56, 61.58, 67.21, 67.54, 72.57, 123.43, 135.17; HR-ESI-MS: 230.1747 ($\text{C}_{12}\text{H}_{24}\text{O}_3\text{N}^+$, $[\text{M}+\text{H}]^+$; calcd 230.1751).

3i: White powder: Yield = 44 mg (88%); $[\alpha]_{\text{D}}^{23} +2$ (c 1.0, MeOH); ^1H -NMR (400 MHz, CD_3OD): δ 1.00-1.09 (2H, CyHe), 1.18-1.38 (3H, CyHe), 1.69-1.86 (6H, CyHe), 2.95

(d, 2H, $J = 7.2$ Hz, H-1'), 3.61 (dd, 1H, $J_{3,4} = 4.0$, $J_{2,3} = 9.2$ Hz, H-3), 3.71 (br d, 1H, $J_{1,2} = 7.6$ Hz, H-1), 3.97 (dd, 1H, $J_{1,2} = 7.6$, $J_{2,3} = 9.2$ Hz, H-2), 4.26 (dd, 1H, $J_{3,4} = 4.4$, $J_{4,5} = 4.4$ Hz, H-4), 5.81 (dd, 1H, $J_{1,6} = 2.4$, $J_{5,6} = 10.0$ Hz, H-6). 6.12 (ddd, 1H, $J_{1,5} = 2.0$, $J_{4,5} = 4.8$, $J_{5,6} = 10.3$ Hz, H-5); $^{13}\text{C-NMR}$ (100 MHz, CD_3OD): δ 26.52, 26.55, 26.98, 31.44, 31.54, 36.41, 51.56, 61.47, 67.20, 6.66, 72.56, 123.42, 135.04; HR-ESI-MS: 242.1748 ($\text{C}_{13}\text{H}_{24}\text{O}_3\text{N}^+$, $[\text{M}+\text{H}]^+$; calcd 242.1751).

3m: Amorphous white powder: Yield = 45 mg (100%); $[\alpha]_{\text{D}}^{23} +13$ (c 1.0, MeOH); $^1\text{H-NMR}$ (400 MHz, CD_3OD): δ 0.97 (d, 6H, $J_{3',4'} = 6.4$ Hz, H-4'), 1.59-1.74 (3H, H-2' and H-3'), 3.11-3.16 (m, 2H, H-1'), 3.61 (dd, 1H, $J_{3,4} = 4.0$, $J_{2,3} = 9.2$ Hz, H-3), 3.71 (br d, 1H, $J_{1,2} = 7.6$ Hz, H-1), 3.98 (dd, 1H, $J_{1,2} = 7.6$, $J_{2,3} = 9.2$ Hz, H-2), 4.26 (dd, 1H, $J_{3,4} = 4.4$, $J_{4,5} = 4.4$ Hz, H-4), 5.85 (dd, 1H, $J_{1,6} = 2.4$, $J_{5,6} = 10.0$ Hz, H-6). 6.12 (ddd, 1H, $J_{1,5} = 2.4$, $J_{4,5} = 5.0$, $J_{5,6} = 10.2$ Hz, H-5); $^{13}\text{C-NMR}$ (100 MHz, CD_3OD): δ 22.56, 22.59, 27.21, 35.99, 44.75, 61.19, 67.15, 68.12, 72.48, 124.54, 134.73; HR-ESI-MS: 216.1593 ($\text{C}_{11}\text{H}_{22}\text{O}_3\text{N}^+$, $[\text{M}+\text{H}]^+$; calcd 216.1594).

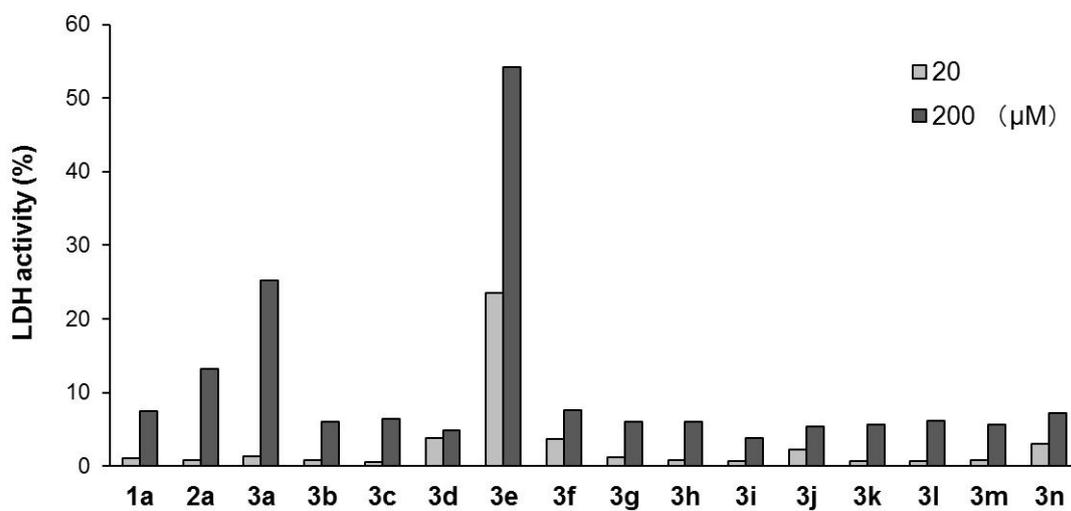
3n: White powder: Yield = 43 mg (84 %); $[\alpha]_{\text{D}}^{23} +1$ (c 1.0, MeOH); $^1\text{H-NMR}$ (400 MHz, CD_3OD): δ 3.01-3.06 (m, 2H, H-1'), 3.34-3.39 (m, 2H, H-2'), 3.60 (dd, 1H, $J_{3,4} = 4.0$, $J_{2,3} = 9.2$ Hz, H-3), 3.74 (br d, 1H, $J_{1,2} = 7.6$ Hz, H-1), 3.98 (dd, 1H, $J_{1,2} = 7.6$, $J_{2,3} = 9.0$ Hz, H-2), 4.25 (dd, 1H, $J_{3,4} = 4.4$, $J_{4,5} = 4.4$ Hz, H-4), 5.83 (dd, 1H, $J_{1,6} = 2.4$, $J_{5,6} = 10.0$ Hz, H-6). 6.12 (ddd, 1H, $J_{1,5} = 2.4$, $J_{4,5} = 4.4$, $J_{5,6} = 10.1$ Hz, H-5), 7.24-7.36 (m, 5H, Ph); $^{13}\text{C-NMR}$ (100 MHz, CD_3OD): δ 33.41, 47.32, 61.17, 67.14, 68.20, 72.46, 123.26, 128.29, 129.76, 129.98, 135.02, 137.73; HR-ESI-MS: 250.1434 ($\text{C}_{14}\text{H}_{20}\text{O}_3\text{N}^+$, $[\text{M}+\text{H}]^+$; calcd 250.1438).

3. IC₅₀ values toward commercially available glycosidases.

Compd	IC ₅₀ (μM)	
	β-galactosidase (bovine liver)	β-glucosidase (almond)
3a	2.0	1.9
3b	79	48
3c	25	13
3d	7.2	0.62
3e	0.70	1.5
3f	800	530
3g	310	5.2
3h	26	31
3i	12	0.58
3j	180	1.7
3k	>1000	>1000
3l	160	710
3m	8.9	24
3n	5.3	1.6

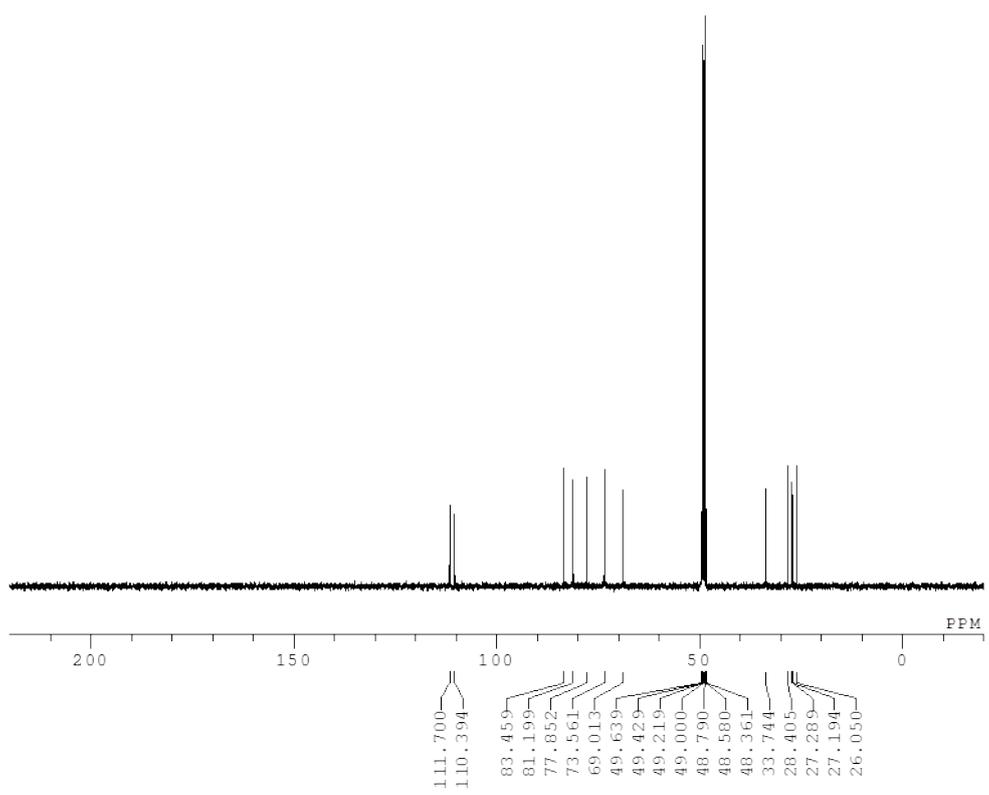
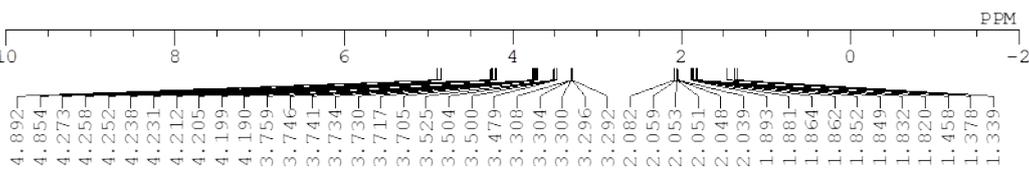
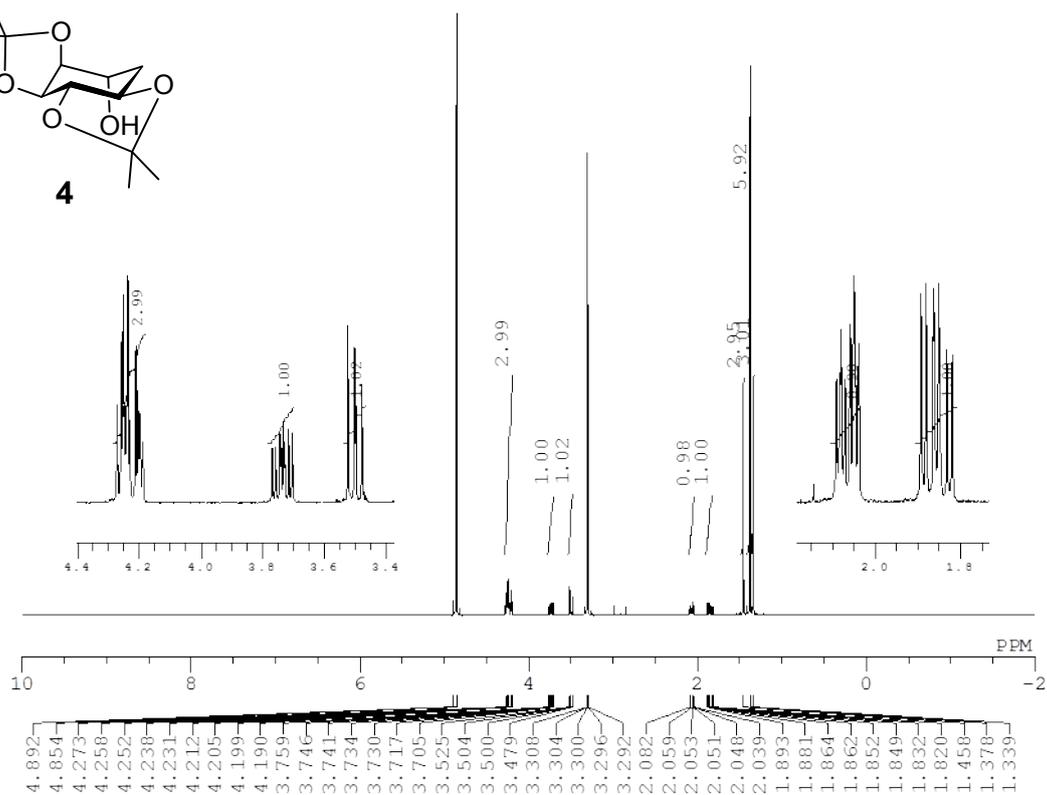
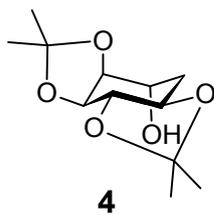
Table S1. Inhibition activities of the derivatives for β-galactosidase (bovine liver) and β-glucosidase (almond).

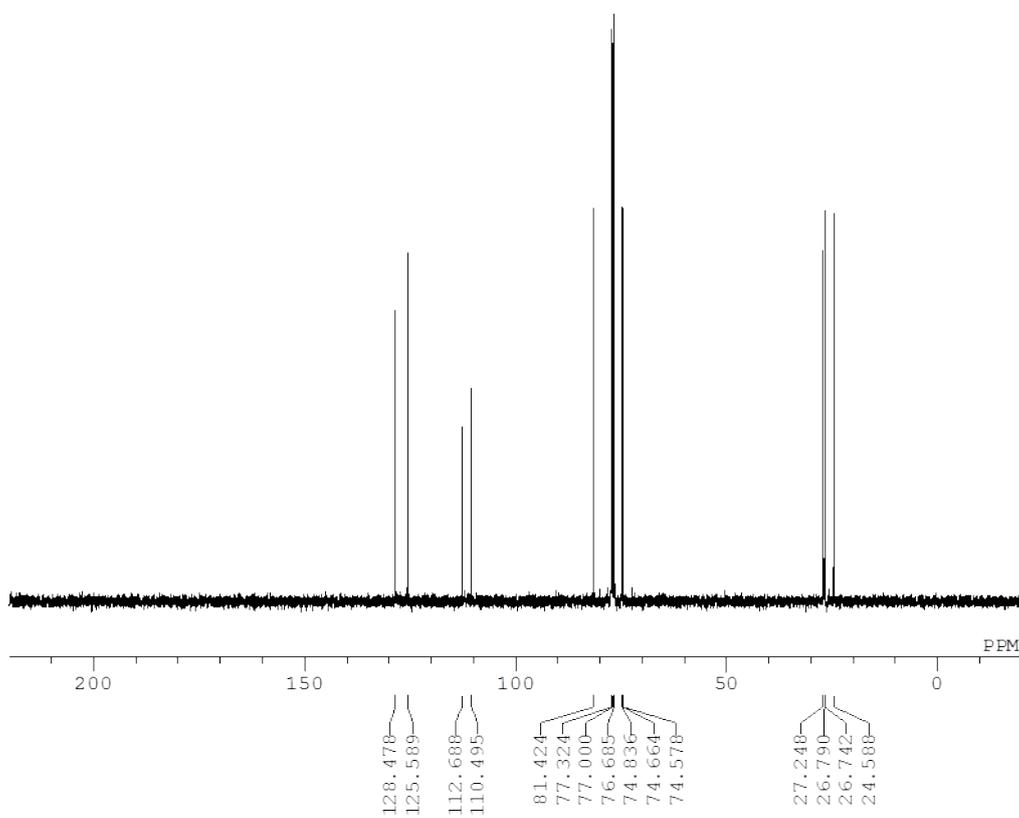
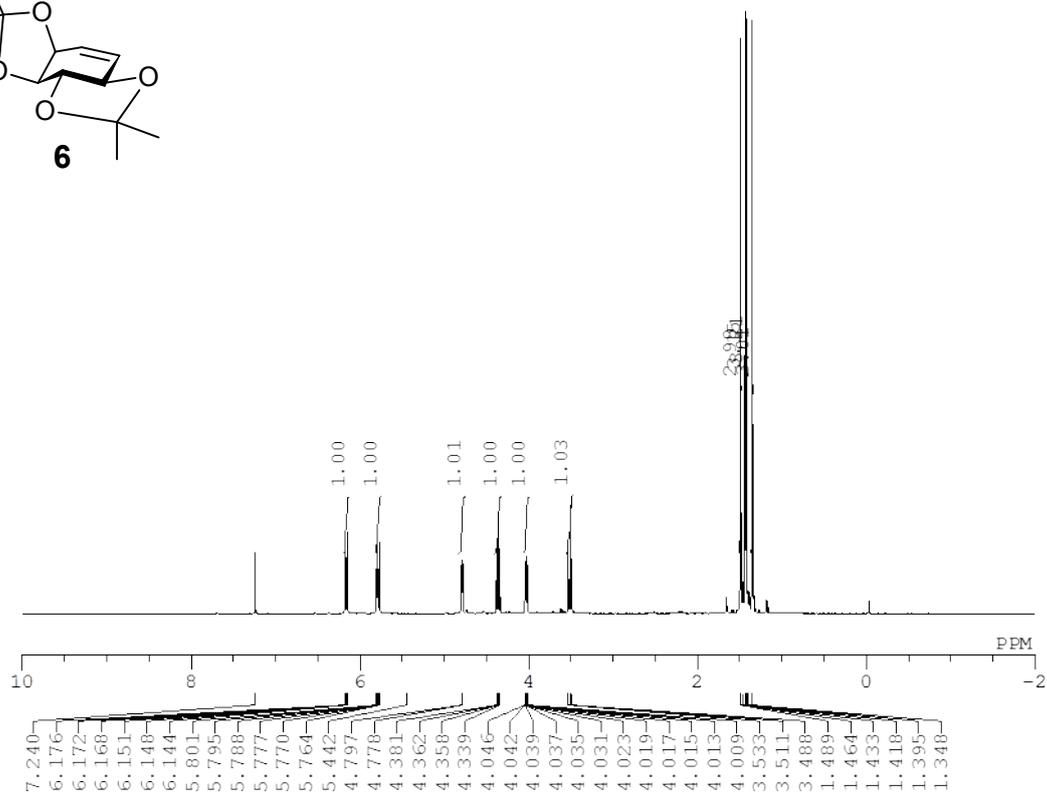
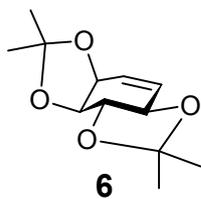
4. Cytotoxic effect of conduramine F-4 derivatives

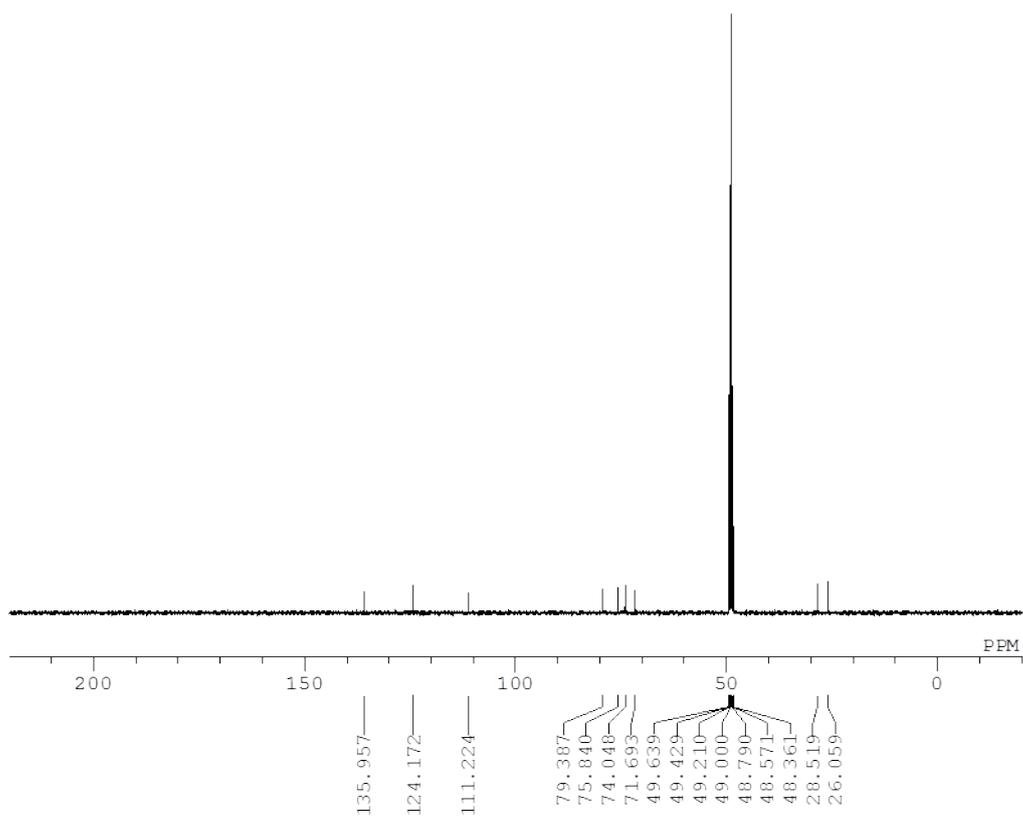
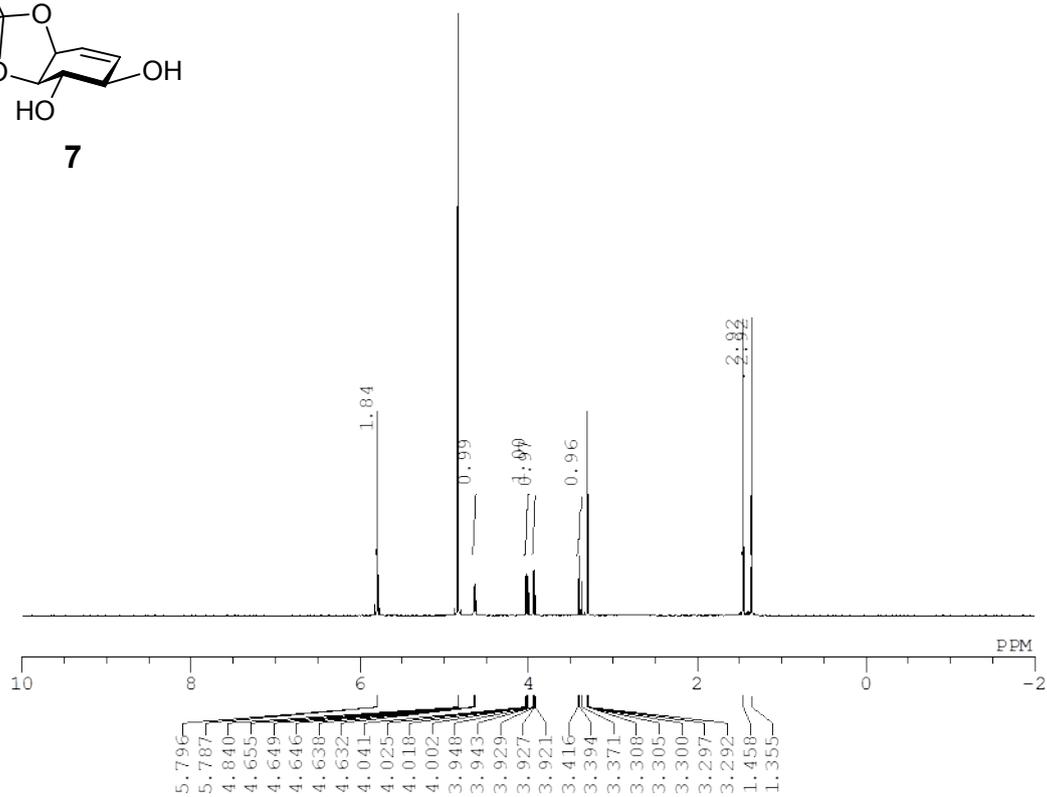
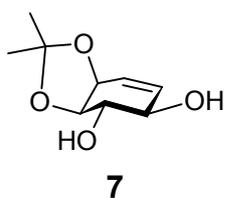


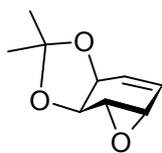
Graph S1. Cytotoxic effect of conduramine F-4 derivatives on cultured human normal fibroblasts (LDH assay).

5. Copies of the ^1H and ^{13}C NMR spectra for the Synthetic Compounds.









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