Supporting Information

Potent Aminocyclitol Glucocerebrosidase Inhibitors are Subnanomolar Pharmacological Chaperones for Treating Gaucher Disease

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Chemistry: General Methods
Solvents were distilled prior to use and dried by standard methods. FT-IR spectra are reported in cm\(^{-1}\). \(^1\)H and \(^{13}\)C NMR spectra were obtained in CDCl\(_3\), CD\(_3\)OD or CD\(_3\)COCD\(_3\) solutions at 500 MHz (for \(^1\)H) and 100 MHz (for \(^{13}\)C). Chemical shifts (\(\delta\)) are reported in ppm relative to the singlet at 7.26 ppm of CDCl\(_3\) for \(^1\)H and in ppm relative to the center line of a triplet at 77.16 ppm of CDCl\(_3\) for \(^{13}\)C. Optical rotations were measured with a Perkin-Elmer Model 341 polarimeter, and specific rotations are reported in \(10^{-1}\) deg cm\(^2\) g\(^{-1}\). The HRMS spectra were recorded on a Waters LCT Premier Mass spectrometer.

Synthesis and Compound Characterization
The following compounds were synthesized according to literature procedures:

\((1SR,2SR,3SR,4RS,5RS,6SR)-2-Azido-3,4,5,6-tetrakis(benzyloxy)cyclohexanol\) 10,
\((1R,2R,3S,4R,5R,6S)-2-Azido-3,4,5,6-tetrakis(benzyloxy)cyclohexanol\) 11,
\((1S,2R,3S,4R,5R,6S)-2-Azido-3,4,5,6-tetrakis(benzyloxy)cyclohexanol\) 14,
\((1RS,2SR,3SR,4RS,5RS,6SR)-2-amino-3,4,5,6-tetrakis(benzyloxy)cyclohexanol\) 17,
\((1S,2R,3S,4R,5R,6S)-2-Amino-3,4,5,6-tetrakis(benzyloxy)cyclohexanol\) 20.

General Procedure for the Alkylation of Azido Alcohols 11 and 14: Synthesis of 12, 13, and 15.
To a solution of 11 or 14 (125 mg, 0.22 mmol) in DMF (4 mL) was added NaH (15 mg, 60\% dispersion in mineral oil, 0.38 mmol) at 0 °C and stirred for 15 min. Then, nonyl iodide or methyl iodide (0.44 mmol) was added, and the mixture was stirred at 0 °C for additional 30 min. The reaction was quenched by addition of few drops of water. The mixture was diluted with 40 mL of Et\(_2\)O and 40 mL of water. The organic layer was separated and the aqueous layer extracted with Et\(_2\)O (3 x 40 mL). The combined organic layers were dried over MgSO\(_4\), filtrated, and concentrated under reduced pressure. The residue was purified by column chromatography (20:1 to 6:1 hexane/EtOAc gradient) to give the desired products.

\(((1S,2R,3R,4S,5R,6R)-5-azido-6-methoxycyclohexane-1,2,3,4-tetrayl)tetrakis(oxy)tetrakis(methylene)tetrabenzene\) (12)
Following the general procedure, 722 mg (1.24 mmol, 93\%) of 12 were obtained from 762 mg (1.34 mmol) of azido alcohol 11. \([\alpha]_{D}^{25}+6.0\) (c 0.5, CHCl\(_3\)); IR (film): \(\nu = \) 3090, 3066, 3026, 2922, 2857, 2106, 1499, 1454, 1360, 1137, 1064, 1028, 735, 697 cm\(^{-1}\); \(^1\)H NMR (\(\delta\), 500 MHz, CDCl\(_3\)): 3.08 (t, 1H, \(J=9.6\) Hz), 3.32 (t, 1H, \(J=9.6\) Hz), 3.42 (t, 1H, \(J=10.0\) Hz), 3.45-3.52 (m, 2H), 3.57 (t, 1H, \(J=9.3\) Hz), 3.70 (s, 3H), 4.82-4.93 (m, 8H), 7.26-7.35 (m, 20H); \(^{13}\)C NMR (\(\delta\), 100 MHz, CDCl\(_3\)): 61.5, 66.7, 76.08, 76.10, 76.14, 81.0, 82.5, 83.3, 83.4 (2), 127.9-128.6, 137.9, 138.3, 138.4. HRMS calculated for C\(_{35}\)H\(_{37}\)N\(_3\)O\(_5\)Na: 602.2631 \([M+Na]^+\). Found: 602.2629.

\(((1S,2R,3R,4S,5R,6R)-5-azido-6-(nonyloxy)cyclohexane-1,2,3,4-tetrayl)tetrakis(oxy)tetrakis(methylene)tetrabenzene\) (13)
Following the general procedure, 145 mg (0.21 mmol, 93\%) of 13 were obtained from 125 mg (0.22 mmol) of azido alcohol 11. \([\alpha]_{D}^{25}+9.1\) (c 1.0, CHCl\(_3\)); IR (film): \(\nu = \) 3089, 3064, 3026, 2925, 2854, 2102, 1497, 1454, 1359, 1137, 1069, 1028, 733, 696 cm\(^{-1}\); \(^1\)H NMR (\(\delta\), 500 MHz, CDCl\(_3\)): 0.89 (t, 3H, \(J=6.9\) Hz), 3.32 (t, 1H, \(J=9.6\) Hz), 3.42 (t, 1H, \(J=10.0\) Hz), 3.45-3.52 (m, 2H), 3.57 (t, 1H, \(J=9.3\) Hz), 3.70 (s, 3H), 4.82-4.93 (m, 8H), 7.26-7.35 (m, 20H); \(^{13}\)C NMR (\(\delta\), 100 MHz, CDCl\(_3\)): 61.5, 66.7, 76.08, 76.10, 76.14, 81.0, 82.5, 83.3, 83.4 (2), 127.9-128.6, 137.9, 138.3, 138.4. HRMS calculated for C\(_{35}\)H\(_{37}\)N\(_3\)O\(_5\)Na: 602.2631 \([M+Na]^+\). Found: 602.2629.
Following the general procedure, 82 mg (0.14 mmol, 71%) of 15 were obtained from 115 mg (0.20 mmol) of azido alcohol 14. [α]25D +5.2 (c 1.0, CHCl3); IR (film): ν = 3090, 3031, 2925, 2854, 2102, 1497, 1453, 1361, 1069, 1028, 735, 696 cm−1; 1H NMR (δ, 500 MHz, CDCl3): 3.23 (dd, 1H, J = 10.4, 2.1 Hz), 3.44 (dd, 1H, J = 9.8, 2.1 Hz), 3.49-3.55 (m, 2H), 3.70 (s, 3H), 3.98 (t, 1H, J = 9.8 Hz), 4.01 (t, 1H, J = 9.6 Hz), 4.70-4.93 (m, 8H), 7.30-7.35 (m, 20H); 13C NMR (δ, 100 MHz, CDCl3): 61.6, 63.4, 73.3, 75.8, 75.9, 76.2, 78.8, 80.3, 81.4, 81.6, 84.4, 127.7-128.6, 138.03, 138.06, 138.61, 138.65. HRMS calculated for C35H37N3O5 Na: 602.2631 [M+Na]+. Found: 602.2625.

Reduction of Azides 12, 13, and 15: Synthesis of Amines 18, 19, and 21.

**General method:** A solution of the starting azide (145 mg, 0.21 mmol) in anhydrous THF (4 mL) was added dropwise under argon a solution of LiAlH4 (18 mg, 0.42 mmol) in anhydrous THF (4 mL) at 0 ºC. After stirring for 3 h at room temperature, the mixture was cooled down to 0 ºC, quenched with dropwise addition of aqueous saturated Na2SO4 solution. The solution was diluted with EtOAc, dried over MgSO4, and filtered through a plug of Celite, which was washed three times with EtOAc. The combined filtrates and washings were concentrated under reduced pressure to afford the corresponding amines.

**1R,2S,3R,4R,5S,6R-2,3,4,5-tetrakis(benzyloxy)-6-methoxycyclohexanamine (18)**

Obtained in 95% yield (655 mg, 1.18 mmol) from 722 mg (1.25 mmol) of azide 12 as a white solid. [α]25D +9.7 (c 1.0, CHCl3); IR (film): ν = 3092, 3064, 3030, 2904, 2853, 1497, 1454, 1356, 1130, 1063, 1043, 1027, 734, 695 cm−1; 1H NMR (δ, 500 MHz, CDCl3): 1.81 (br s, 2H), 2.90 (t, 1H, J =10.0 Hz), 3.09 (t, 1H, J =9.5 Hz), 3.36 (t, 1H, J =9.4 Hz), 3.50-3.64 (m, 3H), 3.68 (s, 3H), 4.70-5.02 (m, 8H), 7.29-7.36 (m, 20H); 13C NMR (δ, 100 MHz, CDCl3): 55.6, 61.6, 75.8, 75.9, 76.0, 76.1, 82.9, 83.5, 84.34, 84.35, 84.39, 127.8-128.7, 138.42, 138.45, 138.50, 138.55. HRMS calculated for C35H40NO5: 554.2906 [M+H]+. Found: 554.2838.

**1R,2S,3R,4R,5S,6R-2,3,4,5-tetrakis(benzyloxy)-6-(nonyloxy)cyclohexanamine (19)**

Obtained in 72% yield (100 mg, 0.15 mmol) from 145 mg (0.21 mmol) of azide 13.

[α]25D +7.6 (c 1.0, CHCl3); IR (film): ν = 3090, 3065, 3033, 2924, 2854, 1497, 1452, 1356, 1130, 1069, 1043, 1024, 732, 696 cm−1; 1H NMR (δ, 500 MHz, CDCl3): 0.90 (t, 3H, J =7.0 Hz), 1.19-1.40 (m, 12H), 1.54-1.67 (m, 2H), 1.90 (br s, 2H), 2.90 (t, 1H, J =10.0 Hz), 3.16 (t, 1H, J =9.4 Hz), 3.35 (t, 1H, J =9.4 Hz), 3.53-3.69 (m, 4H), 3.93-3.97 (m, 1H), 4.71-5.02 (m, 8H), 7.26-7.37 (m, 20H); 13C NMR (δ, 100 MHz, CDCl3): 14.3, 22.8, 26.3, 29.4, 29.6, 29.7, 30.6, 32.0, 55.5, 74.1, 75.85, 75.89, 76.0, 76.1, 82.7, 83.1, 83.6, 84.4 (2), 127.8-128.7, 138.45, 138.47, 138.53. HRMS calculated for C43H56NO5: 666.4158 [M+H]+. Found: 666.4131.
Original text:

(1R,2S,3R,4R,5S,6S)-2,3,4,5-tetrakis(benzyloxy)-6-methoxycyclohexanamine (21)
Obtained in 85% yield (65 mg, 0.12 mmol) from 75 mg (0.13 mmol) of azide 15.

\[ [\alpha]_{D}^{25} -31.2 (c 1.0, CHCl_3); \]

\[ \text{IR (film): } \nu = 3089, 3066, 3033, 2921, 2854, 1496, 1454, 1362, 1090, 1071, 1028, 735, 696 \text{ cm}^{-1}; \]

\[ ^{1}H \text{ NMR (} \delta, 500 \text{ MHz, CDCl}_3): 2.48 (d, 1H, } J = 9.4 \text{ Hz), 3.43-3.50 (m, 3H), 3.63 (s, 3H), 3.71-3.75 (m, 1H), 3.96 (t, 1H, } J = 9.3 \text{ Hz), 4.61-4.93 (m, 8H), 7.24-7.35 (m, 20H); \]

\[ ^{13}C \text{ NMR (} \delta, 100 \text{ MHz, CDCl}_3): 54.3, 61.8, 73.1, 75.7, 75.9, 76.0, 79.9, 82.3, 82.9, 83.3, 85.0, 127.0-128.65, 138.35, 138.67, 138.80, 138.86. \]

HRMS calculated for C_{35}H_{40}NO_5: 554.2906 [M+H]^+. Found: 554.2927.

A solution of the starting amine 17-21 (0.11 mmol) in MeOH (5 mL) under an atmosphere of argon was treated successively with NaBH_3CN (0.23 mmol), AcOH (8 μL) and nonanal (0.12 mmol). After stirring for 4 h at room temperature, the mixture was quenched with water (0.2 mL) and the solvents were removed under reduced pressure. The resulting residue was dissolved in Et_2O (20 ml) and washed with water (15 mL). The aqueous phase was extracted with Et_2O (3 x 20 mL). The combined organic layers were washed with brine and dried over MgSO_4. Filtration and evaporation afforded crude compounds, which were purified as indicated below.

(1R,2S,3R,4R,5S,6R)-2,3,4,5-tetrakis(benzyloxy)-6-(nonylamino)cyclohexanol (23)
According to the general procedure for reductive amination, 49 mg (0.07 mmol, 82%) of 23 were obtained from 49 mg (0.09 mmol) of amino alcohol 17. The compound was purified by flash chromatography (4:1 to 3:1 hexane/EtOAc gradient). IR (film): \[ \nu = 3300, 3085, 3065, 3026, 2947, 2923, 2850, 1496, 1454, 1362, 1086, 1070, 1027 \text{ cm}^{-1}; \]

\[ ^{1}H \text{ NMR (} \delta, 500 \text{ MHz, CDCl}_3): 0.92 (t, 3H, } J = 6.8 \text{ Hz), 1.25-1.38 (m, 12H), 1.46-1.55 (m, 2H), 2.76-2.88 (m, 2H), 3.33-3.37 (m, 1H), 3.51-3.55 (m, 2H), 3.59-3.61 (m, 1H), 3.90 (dt, 1H, } J = 1.8, 9.1 \text{ Hz), 3.97 (dt, 1H, } J = 2.0, 9.0 \text{ Hz), 4.65-4.97 (m, 8H), 7.26-7.41 (m, 20H); \]

\[ ^{13}C \text{ NMR (} \delta, 100 \text{ MHz, CDCl}_3): 14.3, 22.8, 27.4, 29.4, 29.7, 29.8, 30.8, 32.0, 50.8, 57.7, 72.3, 72.6, 75.4, 75.6, 75.7, 81.0, 81.8, 81.9, 83.4, 127.7-128.7, 138.2, 138.7, 138.8. \]

HRMS calculated for C_{43}H_{56}NO_5: 666.4158 [M+H]^+. Found: 666.4180.

(1R,2S,3R,4R,5S,6R)-2,3,4,5-tetrakis(benzyloxy)-6-methoxy-N-nonylcyclohexanamine (24)
According to the general procedure for reductive amination, 64 mg (0.09 mmol, 73%) of 24 were obtained from 72 mg (0.13 mmol) of amine 18. The compound was purified by flash chromatography using a 2:1 mixture of hexane: EtOAc. Oil; \[ [\alpha]_{D}^{25} +13.0 (c 0.3, CHCl_3); \]

\[ \text{IR (film): } \nu = 3089, 3065, 3033, 2924, 2854, 1497, 1454, 1359, 1136, 1064, 1028, 733, 697 \text{ cm}^{-1}; \]

\[ ^{1}H \text{ NMR (} \delta, 500 \text{ MHz, CDCl}_3): 0.89 (t, 3H, } J = 6.99 \text{ Hz), 1.22-1.44 (m, 14H), 2.55 (t, 1H, } J = 10.1 \text{ Hz), 2.73-2.81 (m, 2H), 3.16 (t, 1H, } J = 9.2 \text{ Hz), 3.41 (t, 1H, } J = 9.5 \text{ Hz), 3.49-3.61 (m, 3H), 3.65 (s, 3H), 4.73-5.00 (m, 8H), 7.28-7.35 (m, 20H); \]

\[ ^{13}C \text{ NMR (} \delta, 100 \text{ MHz, CDCl}_3): 14.3, 22.8, 27.4, 29.4, 29.7, 30.7, 32.0, 49.9, 61.2, 62.1, 75.7, 75.91, 75.93, 76.1, 82.0, 83.2, 84.24, 84.44, 84.49, 127.8-128.7, 138.3, 138.47, 138.85. \]

HRMS calculated for C_{44}H_{58}NO_5: 680.4315 [M+H]^+. Found: 680.4221.
According to the general procedure for reductive amination, 74 mg (0.09 mmol, 81%) of 25 were obtained from 77 mg (0.12 mmol) of amine 19. The compound was purified by flash chromatography (20:1 to 6:1 hexane/EtOAc gradient). Oil; [α]_D^25 +5.2 (c 1.0, CHCl₃); IR (film): ν = 3089, 3064, 3030, 2955, 2924, 2854, 1497, 1467, 1454, 1358, 1133, 1064, 1028, 735, 696 cm⁻¹; ¹H NMR (δ, 500 MHz, CDCl₃): 0.90 (dt, 6H, J=6.9, 7.0 Hz), 1.22-1.45 (m, 22H), 1.42-1.45 (m, 2H), 1.55-1.66 (m, 2H), 2.27-2.30 (m, 2H), 2.56 (t, 1H, J=10.0 Hz), 2.76-2.85 (m, 2H), 3.20-3.23 (m, 1H), 3.37-3.44 (m, 1H), 3.51-3.68 (m, 4H), 3.91-3.95 (m, 1H), 4.74-4.98 (m, 8H), 7.27-7.35 (m, 20H); ¹³C NMR (δ, 100 MHz, CDCl₃): 14.3, 22.8, 26.4, 27.4, 29.4, 29.5, 29.7, 30.7, 32.03, 32.05, 50.4, 62.2, 74.0, 75.7, 75.9, 76.0, 76.1, 82.2, 82.6, 83.3, 84.5 (2), 127.79-128.63, 138.4, 138.5, 138.56, 138.59. HRMS calculated for C₅₂H₇₄NO₅: 792.5567 [M+H]^+. Found: 792.5620.

According to the general procedure for reductive amination, 74 mg (0.11 mmol, 90%) of 26 were obtained from 66 mg (0.12 mmol) of amino alcohol 20. The compound was purified by flash chromatography (4:1 to 3:1 hexane/EtOAc gradient). Oil; [α]_D^25 -20.2 (c 1.0, CHCl₃); IR (film): ν = 3300, 3087, 3058, 3028, 2947, 2924, 2853, 1496, 1454, 1362, 1086, 1070, 1028 cm⁻¹; ¹H NMR (δ, 500 MHz, CDCl₃): 0.93 (t, 3H, J=7.0 Hz), 1.28-1.44 (m, 14H), 2.41-2.45 (m, 1H), 2.47 (dd, 1H, J=2.4, 10.0 Hz), 2.61-2.66 (m, 1H), 3.47 (dd, 1H, J=2.8, 9.6 Hz), 3.59 (t, 1H, J=9.4 Hz), 3.70 (t, 1H, J=9.7 Hz), 4.08 (t, 1H, J=9.5 Hz), 4.15 (t, 1H, J=2.5 Hz), 4.61-5.05 (m, 8H), 7.30-7.46 (m, 20H); ¹³C NMR (δ, 100 MHz, CDCl₃): 14.3, 22.8, 27.3, 29.4, 29.6, 29.7, 30.1, 32.0, 47.3, 60.9, 65.4, 72.6, 75.8, 76.1, 80.1, 81.2, 81.7, 85.0, 127.7-128.7, 138.4, 138.5, 138.7, 138.9. HRMS calculated for C₄₃H₅₆NO₅: 666.4158 [M+H]^+. Found: 666.4175.

According to the general procedure for reductive amination, 62 mg (0.09 mmol, 80%) of 27 were obtained from 63 mg (0.11 mmol) of amine 21. The compound was purified by flash chromatography (20:1 to 5:1 hexane/EtOAc gradient). Oil; [α]_D^25 -12.9 (c 1.0, CHCl₃); IR (film): ν = 3089, 3068, 3058, 2947, 2924, 2853, 1496, 1454, 1362, 1086, 1070, 1028 cm⁻¹; ¹H NMR (δ, 500 MHz, CDCl₃): 0.88 (t, 3H, J=5.9 Hz), 1.24-1.35 (m, 12H), 1.44-1.52 (m, 2H), 2.50-2.90 (m, 3H), 3.54 (t, 1H, J=8.7 Hz), 3.60-3.73 (m, 5H), 3.87 (t, 1H, J=9.5 Hz), 3.99-4.01 (m, 1H), 4.72-4.93 (m, 8H), 7.22-7.42 (m, 20H); ¹³C NMR (δ, 100 MHz, CDCl₃): 14.4, 23.3, 28.0, 29.9, 30.1, 30.3, 30.4, 32.6, 48.4, 61.6, 61.7, 73.1, 75.8, 75.9, 76.0, 82.6 (2), 83.2, 85.5, 128.0-129.1, 139.9, 140.2, 140.3 (2). HRMS calculated for C₄₄H₅₈NO₅: 680.4315 [M+H]^+. Found: 680.4346.

A solution of amino alcohol 20 (50 mg, 0.09 mmol) in methanol (5 mL) under an atmosphere of argon were treated successively with NaBH₃CN (29 mg, 0.46 mmol), AcOH (8 µL) and nonanal (39 µL, 0.23 mmol). After stirring for 4 h at room temperature, the mixture was quenched with water (0.2 mL) and the solvents were removed under reduced pressure. The resulting residue was dissolved in Et₂O (20 mL) and washed with water (15 mL). The aqueous phase was extracted with Et₂O (3 x 20 mL). The combined organic layers were washed with brine, dried over MgSO₄, and concentrated under reduced pressure to give a yellow oil, which was purified by flash chromatography using a mixture of hexane/EtOAc (20:1). Product 29 was obtained as a colorless
oil (60 mg, 0.07 mmol, 81%); \([\alpha]_D^{25} -28.5\) (c 1.0, CHCl_3); IR (film): \(\nu = 3085, 3065, 3026, 2958, 2938\)

Synthesis of Aminocyclitols 2-6, 8, and 9 by Hydrogenolysis Using Pd/C Catalyst

**General method:** In a glass pressure flask, the benzylated amino or azido compound (0.1 mmol) was dissolved in a mixture of THF (3 mL) and concentrated HCl (4 drops). Pd/C (40 mg, 5-15% Pd on activated C, water-wet) was then added. The flask was repeatedly filled and evacuated with hydrogen and vigorously stirred at room temperature for 24 h under H_2 (2 atm). The reaction mixture was next filtered through a plug of Celite to separate the catalyst, and the filter was washed three times with MeOH. The combined filtrates and washings were concentrated under reduced pressure to give the desired products.

(1R,2S,3R,4R,5S,6R)-6-(nonylamino)cyclohexane-1,2,3,4,5-pentaol hydrochloride (2)

Obtained in 91% yield (22 mg, 0.06 mmol) as a white solid from 46 mg (0.07 mmol) of 23. ^1^H NMR (\(\delta\), 500 MHz, CD_3OD): 0.90 (t, 3H, \(J=6.9\) Hz), 1.24-1.45 (m, 12H), 1.70-1.80 (m, 2H), 3.14-3.26 (m, 3H), 3.45-3.51 (m, 2H), 3.62-3.68 (m, 1H); ^13^C NMR (\(\delta\), 100 MHz, CD_3OD): 14.4, 23.6, 26.4, 27.5, 30.2, 30.3, 30.4, 32.9, 52.2, 63.0, 69.8 (2), 73.9 (2), 74.9. HRMS calculated for C_{15}H_{32}NO_5: 306.2280 [M+H]^+. Found: 306.2275.

(1S,2S,3S,4R,5S,6S)-6-(nonylamino)cyclohexane-1,2,3,4,5-pentaol hydrochloride (3)

Obtained in 92% yield (33 mg, 0.10 mmol) as a white solid from 71 mg (0.11 mmol) of 26. ^1^H NMR (\(\delta\), 500 MHz, CD_3OD): 0.90 (t, 3H, \(J=6.7\) Hz), 1.24-1.45 (m, 12H), 1.64-1.83 (m, 2H), 3.04-3.18 (m, 3H), 3.18-3.26 (m, 2H), 3.34-3.37 (m, 1H), 3.44 (t, 1H, \(J=8.9\) Hz), 3.49-3.54 (m, 1H), 3.66 (s, 3H); ^13^C NMR (\(\delta\), 100 MHz, CD_3OD): 14.5, 23.7, 27.0, 27.8, 30.3, 30.4, 30.5, 33.0, 46.5, 61.1, 68.0, 70.9, 73.5, 73.6, 76.8. HRMS calculated for C_{15}H_{32}NO_5: 306.2280 [M+H]^+. Found: 306.2269.

(1S,2R,3S,4R,5R,6S)-5-methoxy-6-(nonylamino)cyclohexane-1,2,3,4-tetraol hydrochloride (4)

Obtained in 94% yield (25 mg, 0.07 mmol) as a white solid from 51 mg (0.07 mmol) of 24. ^1^H NMR (\(\delta\), 500 MHz, CD_3OD): 0.90 (t, 3H, \(J=6.9\) Hz), 1.31-1.43 (m, 12H), 1.70-1.75 (m, 2H), 3.03 (t, 1H, \(J=10.8\) Hz), 3.08-3.17 (m, 2H), 3.18-3.26 (m, 2H), 3.34-3.37 (m, 1H), 3.44 (t, 1H, \(J=8.9\) Hz), 3.49-3.54 (m, 1H), 3.66 (s, 3H); ^13^C NMR (\(\delta\), 100 MHz, CD_3OD): 14.5, 23.7, 27.3, 27.6, 30.2, 30.4, 30.5, 33.0, 46.0, 60.9, 61.7, 69.8, 75.3, 76.3, 77.4, 79.0. HRMS calculated for C_{16}H_{34}NO_5: 320.2437 [M+H]^+. Found: 320.2433.
(1R,2S,3R,4S,5S,6R)-5-(nonylamino)-6-(nonyloxy)cyclohexane-1,2,3,4-tetraol hydrochloride (5)
Obtained in 89% yield (32 mg, 0.07 mmol) as a white solid from 62 mg (0.08 mmol) of 25. 
[α]D 25 +9.3 (c 0.9, CH3OH); 1H NMR (δ, 500 MHz, CD3OD): 0.90 (dt, 6H, J=7.0, 6.9 Hz), 1.31-1.43 (m, 24H), 1.60-1.76 (m, 4H), 3.00-3.12 (m, 2H), 3.16-3.21 (m, 2H), 3.24 (t, 1H, J=9.1 Hz), 3.40-3.48 (m, 3H), 3.60-3.65 (m, 1H); 13C NMR (δ, 100 MHz, CD3OD): 14.5, 23.8, 27.3, 27.4, 27.7, 30.3, 30.4, 30.5, 30.6, 30.8, 31.5, 33.06, 33.12, 46.8, 62.0, 70.0, 74.1, 75.4, 76.3, 77.6, 78.0. HRMS calculated for C24H50NO5: 432.3689 [M+H]+. Found: 432.3675.

(1S,2R,3S,4R,5S,6S)-5-methoxy-6-(nonylamino)cyclohexane-1,2,3,4-tetraol hydrochloride (6)
Obtained in 89% yield (26 mg, 0.08 mmol) as a white solid from 55 mg (0.08 mmol) of 27. 
[α]D 25 -15 (c 0.9, CH3OH); 1H NMR (δ, 500 MHz, CD3OD): 0.90 (t, 3H, J=6.8 Hz), 1.27-1.43 (m, 12H), 1.60-1.85 (m, 2H), 3.00-3.24 (m, 4H), 3.50-3.60 (m, 2H), 3.65-3.72 (m, 4H), 3.91-3.97 (m, 1H); 13C NMR (δ, 100 MHz, CD3OD): 14.4, 23.7, 27.0, 27.7, 30.3, 30.4, 30.5, 33.0, 47.0, 60.9, 62.0, 71.2, 73.6, 74.8, 77.0, 78.0. HRMS calculated for C16H34NO5: 320.2437 [M+H]+. Found: 320.2433.

(1R,2S,3R,4S,5S,6S)-5-amino-6-(nonyloxy)cyclohexane-1,2,3,4-tetraol hydrochloride (8)
Obtained in 90% yield (10 mg, 0.03 mmol) as a white solid from 21 mg (0.03 mmol) of 16. 
[α]D 25 -11.6 (c 0.5, CH3OH); 1H NMR (δ, 500 MHz, CD3OD): 0.90 (t, 3H, J=6.8 Hz), 1.25-1.40 (m, 12H), 1.55-1.70 (m, 2H), 3.10 (d, 1H, J=10.5 Hz), 3.19 (t, 1H, J=9.1 Hz), 3.45-3.53 (m, 1H), 3.54-3.70 (m, 3H), 3.80-3.87 (m, 4H), 3.98-4.05 (m, 1H); 13C NMR (δ, 100 MHz, CD3OD): 14.4, 23.7, 27.1, 30.5, 30.7, 30.8, 31.2, 33.1, 55.2, 71.5, 73.9, 74.6, 75.1, 77.0, 78.7. HRMS calculated for C15H32NO5: 306.2280 [M+H]+. Found: 306.2265.

(1S,2S,3S,4R,5S,6S)-6-(dinonylamino)cyclohexane-1,2,3,4,5-pentaol hydrochloride (9)
Obtained in 86% yield (21 mg, 0.04 mmol) as a white solid from 40 mg (0.05 mmol) of 29. 
[α]D 25 -15 (c 1.0, CH3OH); 1H NMR (δ, 500 MHz, CD3OD): 0.90 (t, 6H, J=6.5 Hz), 1.21-1.47 (m, 24H), 1.66-1.80 (m, 4H), 3.16-3.35 (m, 4H), 3.40-3.50 (m, 2H), 3.53-3.60 (m, 2H), 4.04 (dd, 1H, J=8.9, 10.6 Hz), 4.24-4.28 (m, 1H); 13C NMR (δ, 100 MHz, CD3OD): 14.4, 23.7, 25.8, 26.7, 27.8, 27.9, 30.20, 30.23, 30.3, 30.5, 33.0, 54.0, 54.9, 64.5, 68.5, 69.2, 73.7, 73.8, 77.2. HRMS calculated for C24H50NO5: 432.3689 [M+H]+. Found: 432.3686.

Biological Assays
Materials: The glycosidases α-glucosidase (from baker’s yeast and rice), β-glucosidase (from almond), β-galactosidase (from bovine liver), and α-galactosidase (from green coffee beans) that were used in the inhibition studies, as well as 4-methylumbelliferyl-β-D-glucoside and the corresponding p-nitrophenyl glycoside substrates, were purchased from Sigma-Aldrich. Imiglucerase (Cerezyme; recombinant human β-glucocerebrosidase analogue) was kindly provided by Genzyme.
Recombinant GCase Inhibition Assay

**Figure S1.** Lineweaver-Burk plot for the inhibition of Imiglucerase by 2 at pH 5.2 ($K_i=17.9 \mu M$).

**Figure S2.** Lineweaver-Burk plot for the inhibition of Imiglucerase by 3 at pH 5.2 ($K_i=32 \text{ nM}$).

**Figure S3.** Lineweaver-Burk plot for the inhibition of Imiglucerase by 4 at pH 5.2 ($K_i=1.58 \mu M$).
Figure S4. Lineweaver-Burk plot for the inhibition of Imiglucerase by 5 at pH 5.2 ($K_i=0.28 \mu M$).

Figure S5. Lineweaver-Burk plot for the inhibition of Imiglucerase by 6 at pH 5.2 ($K_i=53 \text{ nM}$).

Figure S6. Lineweaver-Burk plot for the inhibition of Imiglucerase by 8 at pH 5.2 ($K_i=0.13 \mu M$).
Figure S7. Lineweaver-Burk plot for the inhibition of Imiglucerase by 9 at pH 5.2 ($K_i = 0.82$ µM).

**Inhibition Assay against Commercial Glycosidases.** The enzyme assay methods were similar to those previously reported. Commercial enzyme solutions were prepared with the appropriate buffer and incubated in 96-well plates at 37 °C without (control) or with inhibitor for 5 min. After addition of the corresponding substrate solution, incubations were prolonged for different time periods: 3 min for $\beta$-glucosidase (from almond) and $\alpha$-glucosidase (from baker’s yeast), 5 min for $\beta$-galactosidase and 10 min for $\alpha$-glucosidase (from rice), 13 min for $\alpha$-galactosidase and stopped by addition of Tris solution (50 µL, 1M) or Na$_2$CO$_3$ (180 µL, 1M), depending on the enzymatic inhibition assay. The amount of $p$-nitrophenol formed was determined at 405 nm with a SpectraMax M5 (Molecular Devices Corporation) spectrophotometer. For $\alpha$-glucosidase (from rice), the activity was determined with $p$-nitrophenyl-$\alpha$-D-glucopyranoside (1 mM) in sodium acetate buffer (50 mM, pH 5.0). For $\alpha$-glucosidase (from baker yeast), the activity was determined with $p$-nitrophenyl-$\alpha$-D-glucopyranoside (1 mM) in sodium phosphate buffer (100 mM, pH 7.2). For $\beta$-glucosidase (from almond), the activity was determined with $p$-nitrophenyl-$\beta$-D-glucopyranoside (1 mM) in sodium acetate buffer (100 mM, pH 5.0). $\beta$-galactosidase activity was determined with $p$-nitrophenyl-$\beta$-D-galactopyranoside (1 mM) in sodium phosphate buffer (100 mM, 0.1 mM MgCl$_2$, pH 7.2). $\alpha$-galactosidase activity was determined with $p$-nitrophenyl-$\alpha$-D-galactopyranoside (1 mM) in sodium phosphate buffer (100 mM, pH 6.8). The commercial glycosidase solutions were prepared as follows: $\alpha$-glucosidase (from rice): (NH$_4$)$_2$SO$_4$ suspension (30 µL) in buffer (1.8 mL); $\alpha$-glucosidase (from baker’s yeast): (0.1 mg mL$^{-1}$ buffer); $\beta$-glucosidase (from almond): (0.1 mg mL$^{-1}$ buffer); $\alpha$-galactosidase (from green coffee beans): 7.4 µL in buffer (1.99 mL); $\beta$-galactosidase from bovine liver (0.5 mg mL$^{-1}$ buffer).
**Table S1.** Activity of Compounds against Commercial Glycosidases and Glucosylceramide Synthase (GCS)

<table>
<thead>
<tr>
<th>Compound</th>
<th>α-Glucosidase (baker’s yeast)</th>
<th>α-Glucosidase (rice)</th>
<th>β-Glucosidase (almond)</th>
<th>α-galactosidase (from green coffee beans)</th>
<th>β-galactosidase (from bovine liver)</th>
<th>GCS</th>
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<tr>
<td></td>
<td>% Inhibition (100 µM)</td>
<td>% Inhibition (100 µM)</td>
<td>% Inhibition (100 µM)</td>
<td>% Inhibition (100 µM)</td>
<td>% Inhibition (100 µM)</td>
<td>% Inhibition (250 µM)</td>
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<td>2</td>
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<td>11</td>
<td>0</td>
<td>74 (31)&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>3</td>
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<tr>
<td>4</td>
<td>8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>15&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>74</td>
<td>0&lt;sup&gt;d&lt;/sup&gt;</td>
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<tr>
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<td>0</td>
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<td>0</td>
<td>24</td>
<td>0</td>
<td>80 (0.58)&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
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</table>

<sup>a</sup> IC<sub>50</sub> value in µM. <sup>b</sup>% Inhibition at 800 µM. <sup>c</sup>% Inhibition at 1.1 mM. <sup>d</sup>% Inhibition at 50 µM.

**Glucosylceramide Synthase Assay.** A549 Cells were washed with sodium phosphate (PBS) (10 mM, 137 mM NaCl, pH=7.4) and collected by brief trypsinization. The cells were then washed twice with PBS and resuspended in 50 mM TRIS-HCl buffer (pH 7.4) and 10 mM MgCl<sub>2</sub> by sonication (three times, 30 seconds). The cell lysate (100 µL) was incubated with inhibitor (250 µM or 50 µM final concentration) for 10 min at 37 °C. Then 25 µL of NAD (16 mM in TRIS-HCl, pH 7.4 and 10 mM MgCl<sub>2</sub>), 25 µL of UDP-Glucose (2 mM in TRIS-HCl 50 mM, pH 7.4 and 10 mM MgCl<sub>2</sub>) and 52 µL of NBD C<sub>6</sub>-ceramide complexed to BSA at a 1:1 ratio (20 µM in 50 mM TRIS-HCl buffer, pH=7.4, 10 mM MgCl<sub>2</sub>) were added. After 15-min incubation at 37 °C, the reactions were stopped by adding 800 µL of MeOH and centrifuged at 10000 rpm for 3 min. The supernatant was transferred to HPLC vials. HPLC analyses were performed with a Waters 2690 Alliance System coupled to a Waters 2475 Fluorescence detector (Milford, MA) using a C18-Kromasil column and eluted with 15% water and 85% acetonitrile, both with a 0.1% of trifluoroacetic acid, flowing at 1 mL/min. The detector was set at an excitation wavelength of 465 nm and measure the emission wavelength at 530 nm. Empower Software (Waters Corporation) was utilized for data acquisition and processing.
Cytotoxicity Assay in Wild-Type Human Fibroblasts. Wild-type fibroblasts were cultured in Dulbecco’s modified Eagle’s medium (DMEM, Sigma-Aldrich) supplemented with 10% foetal bovine serum (FBS, Invitrogen) and 1% penicillin-streptomycin (Invitrogen) at 37 °C in 5% CO₂/95% air. Cells used were between the 14th and 30th passage. At the time of the experiments, cells were seeded at a density of 25000 cells per well in 96-well plates. Media were renewed after 24 h and compounds were added to give final concentrations of 300-18 \( \mu \)M or 300-2 \( \mu \)M (for compounds 5, 7, and 9). All compounds were dissolved in DMSO and control experiments were performed with DMSO. Cells were incubated at 37 °C in 5% CO₂ for 24 h. Then, the media were replaced with 100 \( \mu \)L of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution and the mixture was incubated for additional 3 h at 37 °C in 5% CO₂/95% air. The number of viable cells was quantified by the estimation of its dehydrogenase activity, which reduces MTT to water-insoluble formazan, which was dissolved in 100 \( \mu \)L of DMSO and measured at 570 nm with SpectraMax M5 (Molecular Devices Corporation) in 96-well format.

Table S2. Cytotoxicity of Compounds 1-9 in Wild-Type Human Fibroblasts.

<table>
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<th>Cytotoxicity CC₅₀ (( \mu )M)¹</th>
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<tr>
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<td>23.3</td>
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¹Wild-type fibroblasts were treated with different concentrations of compounds for 24 h, and the cytotoxicity was evaluated as described in the experimental procedures. The CC₅₀ values were obtained by regression analysis of the dose-response curves obtained in a single experiment with triplicates.
GCase Inhibition in Intact Human Fibroblasts

Figure S8. GCase inhibition of aminocyclitols 1–6, 8, and 9 in wild-type human fibroblasts after 24h incubation time at the indicated inhibitor concentrations.

Inhibition Assay of Human Lysosomal Glycosidases. Fibroblasts were seeded at a density of 10^5 cells per well in 24-well plates. After 24 h, the media were replaced with fresh media with or without a test compound and incubated at 37 °C in 5% CO₂ for 24 h. The enzyme activity assay was performed after removing media supplemented with the corresponding compound. The monolayers were washed with 100 μL of PBS solution. Then, 80 μL of PBS and 80 μl of 200 mM acetate buffer (pH 4.0) were added to each well. The reactions were started by the addition of 100 μL of substrates (200 mM acetate buffer, pH 4.0) to each well, followed by incubation at 37 °C for 2 h. The substrates were 4-methylumbelliferyl-β-D-glucopyranoside (5 mM, for GCase), 4-methylumbelliferyl-α-D-glucopyranoside (5 mM, for α-glucosidase), 4-methylumbelliferyl-α-D-galactopyranoside (1 mM, for α-galactosidase), and 4-methylumbelliferyl-β-D-galactopyranoside (1 mM, for β-galactosidase). Enzymatic reactions were stopped by lysing the cells with 1.8 ml of glycine/NaOH buffer (100 mM, pH 10.6). Liberated 4-methylumbelliferone was measured (excitation 355 nm, emission 460 nm) with SpectraMax M5 fluorometer (Molecular Devices Corporation) in 24-well format. All determinations were performed in triplicate. Cells used were between the 14th and 30th passages.
Table S3. Inhibitory Activity (% Inhibition) on Lysosomal Human α-Glucosidase, α-Galactosidase, and β-Galactosidase in Wild-Type Human Fibroblasts for compounds 1-9.

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<td>11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>16&lt;sup&gt;b&lt;/sup&gt;</td>
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<sup>a</sup>Incubation for 24 h at 50 µM inhibitor. <sup>b</sup>Toxic at 50 µM. Incubation for 24 h at 5 µM inhibitor.

Inhibition of GCase and GBA2 in Mouse Tissue Homogenates.

Preparation of membrane fractions: Mouse tissues were homogenized in 50 mM potassium phosphate buffer (pH 5.8) using a small dounce homogeniser at a ratio of 1:3 (w/v, tissue:buffer). Homogenates were centrifuged at 13,000 rpm for 20 min at 4°C. The supernatant was removed and the pellet was resuspended in ice-cold 50 mM potassium phosphate buffer, pH 5.8. This procedure was repeated two times. Final membrane pellets were resuspended in 50 mM potassium phosphate buffer (pH 5.8) to give 1.0 mg/mL protein concentration as determined using a BCA assay, and stored at -78°C.

The inhibitory effects of the aminocyclitols on the activity of the non-lysosomal and lysosomal GCase were determined in membrane preparations from different tissues using a method adapted from ref 5.

For the enzyme assays, aliquots (20 µL) of the membrane suspension were incubated at 37 °C without (control) or with inhibitor at a final volume of 25 µL for 15 min at 37 °C. After addition of 15 µL of substrate, the samples were incubated at 37 °C for 2 h. Then, the enzyme reactions were stopped by the addition of 100 µL of glycine/NaOH buffer (100 mM, pH 10.6) and fluorescence was measured (excitation wavelength 355 nm, emission wavelength 460 nm) with a SpectraMax M5 fluorometer (Molecular Devices Corporation) in 96-well format.

The activity of the non-lysosomal GCase in membrane preparations from testes and brain tissues was determined with 3 mM 4-methylumbelliferyl-β-D-glucopyranoside in McIlvaine buffer (pH 5.8) upon preincubation for 30 min at room temperature with 2.5 mM conduritol B epoxide.

The activity of the lysosomal GCase was determined in membrane preparations from liver tissues with 3 mM 4-methylumbelliferyl-β-D-glucopyranoside in McIlvaine buffer (pH 5.2), supplemented with 0.25 % (w/v) sodium taurocholate and 0.1% (v/v) Triton X.
Figure S9. Inhibition of GCase (A) and GBA2 (B) in mouse tissue homogenates. Compounds were assayed at the indicated inhibitor concentrations (µM) and 3 mM substrate. Each bar represents the mean ± SD of two independent experiments with duplicates.
Thermal Stabilization Assay

Figure S10. Relative enzymatic activity after thermal denaturation (48 °C) for 20, 40 and 60 min at the indicated inhibitor concentrations (µM), compared to the corresponding assay at 37 °C. Data for control (C) are obtained as above except that no inhibitor is present.
Figure S11. Stabilization ratio (the ratio of relative enzyme activities inhibitor vs control) of compounds 1-9 after thermal denaturation (48 °C) for 20, 40 and 60 min at the indicated inhibitor concentrations (μM).
Measurement of L444P or N370S GCase Activity in Lymphoblasts Derived from Patients with GD

**Figure S12.** The influence of aminocyclitols 1-9 on GCase activity in N370S lymphoblasts (GM10873) from Gaucher patients. Cells were cultured for 3 days in the absence or presence of increasing concentrations (µM) of the compounds before GCase activity was measured. Experiments were performed in triplicate, and each bar represents the mean ± SD. Enzyme activity is normalized to untreated cells, assigned a relative activity of 1.
Figure S13. The influence of aminocyclitols 1, 3-7, and 8 on GCase activity in L444P lymphoblasts (GM08752) from Gaucher patients. Cells were cultured for 3 days in the absence or presence of increasing concentrations of the compounds before GCase activity was measured. Experiments were performed in triplicate, and each bar represents the mean ± SD. Enzyme activity is normalized to untreated cells, assigned a relative activity of 1.
References

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HPLC Chromatograms

![HPLC Chromatogram](image)

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